Correlation of the Physical Properties of the Carcinogenic Amines with their Carcinogenicity

By

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I

INTRODUCTION

The discovery that certain specific chemicals can cause cancer will probably rank as one of the major achievements of this century. Basic research in cancer has concentrated largely on a study of the aromatic carcinogens—polynuclear hydrocarbons and aromatic amines that can cause cancer in animals. Because of the basic studies carried out with these chemicals on animals, carcinogenesis in human beings is now much better understood. In many industries a high incidence of industrial cancers, such as mulespinners' cancer, shale cancer, pitch and tar cancer, and cancer of the urinary bladder, have been reported in the past (131,146). The incidence of cancer in these industries has been reduced by decreasing the contact of the worker with industrial processes or products in which some aromatic carcinogen was involved.

With the exception of X-rays, ultraviolet light and radioactive radiations, all known cancer initiators are chemicals. But these exceptions are known to cause large changes in the structure of many compounds, so the basis of their carcinogenic activity is probably also chemical in nature. Consequently one can reasonably assume that carcinogenesis involves several key complex chemical reactions in which the carcinogen (or one of its metabolites) and some body chemical (s) are involved. There are several hundred aromatic compounds that can cause cancer. Their close relationship in structure cannot be accidental. It is reasonable to assume that carcinogenesis involves some specific general properties of the aromatic carcinogens. This is borne out by the many examples of activation or deactivation of a molecule by some 'slight' shift, addition or withdrawal of a chemical group. The same type of examples are found is many general organic chemical reactions. Once we know the general specific properties of the aromatic carcinogens then we could figure out what chemical reactions take place during carcinogenesis and what chemicals could interfere with these reactions. With this knowledge progress toward a solution of the primary problem, the prevention and cure of cancer, would be accelerated.

II

THE CARCINOGENIC AMINES

What do the carcinogens have in common? That, I believe, is the crucial question in cancer research today. It is very probable that carcinogenesis is due to some factor (s) common to all the carcinogens, however, the carcinogens can be separated into several diverse groups. The two largest groups are the polynuclear carcinogens and the carcinogenic amines. As so little is known about carcinogenesis, it is evident that each "homogeneous" group will have to be studied separately. Once the physical, chemical and physiological properties of the members of the group are known, then the groups can be compared. Only through such a thorough study can the problem of carcinogenesis be solved.

Boyland (44) has developed the concept of carcinogenophores and auxocarcinogens, these terms being analogous to chromophores and auxochromes. Boyland believes that the carcinogenophoric group is generally an active phenanthrenic double bond or an amine group, while the auxocarcinogens are usually methyl or other electronegative groups. In respect to these carcinogens the physical and chemical properties that they have in common will probably prove of utmost value in any correlation study. On the basis of the known facts, the following generalizations can be made about the carcinogenic amines.

(1) The molecular weights of these carcinogens range from

approximately 140 (as in B-naphthylamine) to 240 (as in 3'-methyl-4-dimethylaminoazobenzene).

(2) The molecule consists of two to three six-membered rings which are fused or in tandem. The molecule is fully aromatic and can assume a coplanar position. Resonance can take place in and between the different rings.

(3) The amine grouping has to be in a position in which it can resonate with the remainder of the molecule. This is usually in a para position and occasionally in the ortho position.

(4) The extended para position is the position that is usually oxidized in metabolism. In some cases the ortho position is attacked.

(5) In the activated p-quinonoid state three to five sets of conjugated double bonds extend from the amine grouping to the extended para position.

A. Naphthalene Group

1. Naphthalene. Quantum mechanics has shown that the number of independent or canonical structures amongst which resonance can occur is given by

$$\frac{n!}{\frac{n}{2}!\left(\frac{n}{2}+1\right)!}$$

where n is the number of non-localized electrons (287). In benzene, besides the electrons forming the sigma bonds, which are localized, there are six pi electrons and thus the number of canonical structures is five. The five structures are the two Kekule and the three Dewar forms. In naphthalene the number of canonical structures is forty two. Of these, three are Kekule. In this series of hydrocarbons (benzene, naphthalene, anthracene, etc.) the number of Kekule structures is one more than the number of rings. The number of structures with elongated pi bonds (Dewar structures) increases considerably as the number of benzene rings in the molecule is increased and it is this factor that is responsible for the gradual increase in reactivity with the size of the molecule. While benzene has a resonance energy of thirty nine kcal. per mole, naphthalene has seventy five and anthracene has one hundred five (230). The configuration of the molecule is affected by this resonance. Because of resonance each bond in the molecule acquires some double bond character which causes all bonds to become coplanar. By means of X-ray studies Robertson (251,252) has verified that naphthalene and anthracene are coplanar.

Studies on the metabolism of naphthalene show that it is converted to 1,2-dihydroxy-1, 2-dihydronaphthalene; the d-rotary form was obtained from the urine of rabbits and the l-rotary form from the urine of rats (39). Young (307) has also reported the isolation of 1-(1-naphthyl) mercapturic acid and another compound, probably monohydroxydihydronaphthalene, which yielded naphthalene on acid hydrolysis. The metabolism of 2-naphthylamine has also been studied. Dobriner and his co-workers (99) isolated 2-acetylamino-7-hydroxynaphthalene and 2-acetylaminonaphthalene from the urine of rabbits treated with 2-naphthylamine. On the other hand, in the dog 2-naphthylamine gives rise to 2-amino-1-naphthol (297). From these facts it seems probable that the large acetylamino group hinders ortho oxidation, while the much smaller amino group may favor it. Recent work seems to bear this out. When 2-naphthylamine was given subcutaneously or by stomach tube to rats, 2-acetylamino-7-hydroxynaphthalene and 2-amino-1-naphthalene sulfuric acid were isolated from the urine (194). When 2-acetylaminonaphthalene was given to rats subcutaneously, the 7-hydroxy metabolite was isolated. When this same compound was given to rats by way of the stomach tube, the starting product and 2-naphthylamine were isolated (194).

In Table I are shown some naphthalene and anthracene derivatives that can cause cancer in animals. 2-Naphthylamine also causes malignant tumors of the bladder in man (19,132). Because the site of action is the bladder, it is very probable that a water-soluble metabolite excreted in the urine is the actual carcinogen. Good evidence has been presented for this viewpoint, for when 2-amino-1-naphthol was directly implanted in a mouse bladder, tumors were produced. The author states that under these conditions this metabolite is as potent as methylcholanthrene (38).

2. Anthracene. The configuration of anthracene is similar to that of naphthalene. Robertson (252) has shown by careful X-ray work that it has a coplanar configuration.

Anthracene is noncarcinogenic (237). When fed to rabbits or rats it is excreted in the urine as 1,2-dihydroxy-1,2-dihydroanthracene (43).

TABLE I

NAPHTHALENE AND ANTHRACENE CARCINOGENS (143)

2-Naphthylamine
1-Hydroxy-2-naphthylamine
2,2'-Diamino-1,1'-dinaphthyl
2,2'-Azonaphthalene
2-(4'-Dimethylaminophenylazo)naphthalene (169)
1-(4'-Dimethylaminophenyl)-2-(1'-naphthyl)ethylene
2-Anthramine

B. Biphenyl Group

1. Biphenyl. Gillam and Hey (124) have shown that in the para series of polyphenyls the wave length maxima increases as the number of phenylene groups increase. On the other hand in the meta series simple conjugation cannot extend past the diphenyl structure. This is borne out by the wave length maxima of the meta series which remains at 253 ± 2 mu from biphenyl to m-ossidiphenyl. These facts show that biphenyl, p-terphenyl, etc. can assume the coplanar configuration. In this case the para quinonoid forms are probably of great importance. In biphenyl the ground state represented primarily by a hybrid of Kekule forms is elevated to the excited electronic state, which is coplanar, upon absorption of a photon. To the extent that there is any repulsion between the ortho hydrogens the energy of the excited state would be a little higher than without this repulsion. This effect goes hand in hand with the belief that the greater the separation of charges the smaller the contribution of this state to the resonance hybrid. The region of absorption in the ultraviolet of a molecule is probably a consequence of the energy difference between the ground state and the excited state. The greater the energy difference involved, the further in the ultraviolet will a compound absorb. Thus the absorption of biphenyl at shorter wave lengths than that of benzene can be explained. This argument leads to the conclusion that restricted rotation can exist in biphenyl. If a number of large ortho groups are inserted, coplanarity cannot occur without a large expenditure of energy, and the resonance which is of importance in the absorption of light, will be

inhibited. This effect is illustrated by the absorption curves of Pickett (233). On the other hand the spectra of 9,10-dihydrophenanthrene and 4,5-methylene-9,10-dihydrophenanthrene (159) in which the ortho-substituted methylene and dimethylene bridges hold the two aromatic rings in or near a coplanar configuration, possess the biphenyl type of spectra. An X-ray examination (97) has shown biphenyl to consist of flat regular hexagons of carbon atoms lying in one plane, with distances between consecutive atoms in each ring from center to center of 1.42 A. This is significantly shorter than the normal single bond length (1.54 A) and constitutes direct evidence that the hydrocarbon is degenerate, with structures between the unperturbed forms represented by the singly linked Kekule individuals on one hand and the activated quinonoid forms on the other hand. From all these facts it is evident that biphenyl can assume a coplanar configuration.

The metabolism of biphenyl (281) demonstrates again the importance of the 4-position. About 25% of injected biphenyl was recovered from rabbit urine chiefly as free 4-hydroxybiphenyl. Benzidine (278), 4-dimethylaminobiphenyl (209), 4-acetylaminobiphenyl (202) and 4,4'-diamino-3,3'-dihydroxybiphenyl (64) have been reported to be carcinogenic to animals. Benzidine has been reported to be carcinogenic to human beings (19).

2. Fluorene. The parent compounds of the carcinogenic amines are all aromatic. For this reason we are concerned with aromatic character. Six pi electrons (the sextuple group) are necessary for this property. The benzene ring has six such unsaturation electrons present.

Pyrroles are very weak bases. This is probably because the salt-forming electrons (the unshared electrons) are necessary for the sextuple group. Pyridines are strong bases because 6 pi electrons are available and the salt-forming electrons are left relatively untouched. In cyclopentadiene 6 pi electrons can be made available because of the hyperconjugation of the methylene group. This is also so in the 5-membered rings of indene and fluorene but to less an extent, for the acidity of the molecule diminishes in indene, and even more so in fluorene (245). Consequently, it is very probable that the 5-membered ring in fluorene has aromatic character. The structure of fluorene has been discussed by the Weistergers and Ray (294). From a study of the stereochemistry of mono and disubstituted 9,9'-spirobifluorenes and on the basis of theoretical considerations they concluded that fluorene was planar. The fact that the absorption spectra of fluorene and biphenyl are fairly similar while the absorption spectra of 2-acetylaminofluorene and 4-acetylaminobiphenyl have a greater similarity indicates that the fluorene molecule, like biphenyl, can assume a coplanar configuration.

Fluorene is not carcinogenic (300). Its metabolism has been studied in the rabbit (223). As in biphenyl the para position was attacked. 2-Hydroxyfluorene was recovered from the urine. Another compound was also recovered. It was thought to be the dihydrate of the glucuronide of 2-hydroxyfluorene. When 2-acetylaminofluorene was fed to rats, 7-hydroxy-2-acetylaminofluorene was found as the principal metabolite (31). This metabolite has been reported to be much less carcinogenic to albino rats than 2-acetylaminofluorene (153). Some of the carcinogenic fluorene compounds are shown in Table II.

TABLE II

RELATIVE CARCINOGENICITY OF FLUORENE DERIVATIVES

1.	2-Diacetylaminofluorene	10
2.	2,7-Bis-acetylaminofluorene	9
3.	2-Acetylaminofluorene	7
4.	2-Aminofluorene	6
5.	2-Nitrofluorene	5
6.	2-Benzoylaminofluorene	3
7.	3-Iodo-2-acetylaminofluorene	4
8.	7-Hydroxy-2-acetylaminofluorene	weak
9.	7-Chloro-2-acetylaminofluorene	0
10.	7-Iodo-2-acetylaminofluorene	0
11.	2-Tosylaminofluorene	0

3. Dibenzofurans and dibenzothiophene. Very little work has been done on the structure of these compounds. It is probable that, like fluorene, they can assume a coplanar configuration. Merkel and Wiegand (203) have stated the belief that aromatic molecules of flat structure tend to show a fine structure in their long wave absorption bands. If the molecule is not flat, this fine structure is not apparent. On this basis dibenzofuran and dibenzothiophene, which show more fine structure than fluorene, would be coplanar.

Christomanos (76) has studied the metabolism of dibenzofuran (diphenylene oxide). After subcutaneous injection of dibenzofuran dissolved in olive oil, rabbits excreted in the urine a considerable amount of hippuric acid and a slightly increased amount of benzoic acid as compared to the control group. The author states that evidently dibenzofuran breaks apart at the O bridge and the nucleus carrying the O then undergoes destruction while the other nucleus is left intact as benzoic acid which conjugates with glycine in the usual manner.

In Table XII 2-acetylaminofluorene is compared with its dibenzothiophene and dibenzofuran analogs.

TABLE XII

RELATIVE CARCINOGENICITY
OF ANALOGS OF 2-ACETYLAMINOFLUORENE (209)

1.	2-Acetylaminofluorene	7
2.	3-Acetylaminodibenzothiophene	6
3.	3-Acetylaminodibenzofuran	3
4.	3-Acetylaminodibenzothiophene-5-oxide	2
5.	4-Dimethylaminobiphenyl	1

Azo-Stilbene Group

1. Stilbene. X-Ray crystallographic data on trans-stilbene have shown that it is a coplanar molecule (253). On the other hand, an examination of a cis-stilbene scalar model will show, just as in the case of cis-azobenzene, that the ortho hydrogen atoms will prevent the coplanarity of the molecule.

Using the Walker rat carcinoma 256 as the biological test object, Haddow *et al* (136) examined a large number of derivatives of stilbene to determine the relationship between the growth-inhibitory effect and chemical constitution. From their work it is apparent that there is a close connection between the lack of inhibitory power and the buckling of the molecule. The carcinogenic 4-dimethylaminostilbene was found to be a very active tumor inhibitor. Derivatives of this compound with substituents on the alpha and beta carbon atoms of the ethylenic double bond interfere with the planarity of the molecule thus decreasing the conjugation resonance of the whole molecule. The absorption spectra of these derivatives show definite hypochromic and hypsochromic effects. This is correlated with the fact that the compounds are non-inhibitory. Bulky alkyl substituents in the 2'-position seem to influence the 4-dimethylaminostilbene molecule in the same manner. The 2'-methyl derivative is strongly active; the 2'-ethyl derivative is weakly active while the 2'-n-propyl and 2'-isopropyl compounds are inactive. The absorption maxima and the extinction coefficient of these chemicals decrease with the decrease in inactivity. The great majority of the active tumor-inhibitors were found to be stilbenes with a basic substituent in the 4-position and the 4'-position unsubstituted. These facts suggest that an unbroken conjugation of an amino grouping with the benzene rings of the stilbene is necessary for activity. This type of conjugation would enable the compound to assume a dipolar quinonoid form. Any interference with the planarity of the molecule would naturally interfere with this type of conjugation. This was also borne out by the inactivity of the non-planar cis derivatives tested. The authors pointed out that the carcinogenic stilbene derivatives, such as 4-aminostilbene, 4-dimethylaminostilbene, 4-acetylaminostilbene, 4-diethylaminostilbene and 2'-methyl-4-dimethylaminostilbene were also strong tumor inhibitors. From this one could reasonably infer that interference with total conjugation and planarity would affect carcinogenicity as it did tumor inhibition. The metabolism of stilbene has been investigated (280,281) and about 37% of the injected stilbene could be accounted for as benzoic acid (34%) and 4,4'-dihydroxystilbene (3%). There is also some evidence to show that the aminostilbenes are in part excreted in the form of their 4'-hydroxy derivatives (136).

2. Azobenzene. The crystal structure and molecular configuration of cis and trans-azobenzene have been determined by Robertson (254). In Fig. 1 are seen A. transazobenzene, B. the hypothetical coplanar cis azobenzene, and C. the experimental cis azobenzene. In B it is seen that the distance between the ortho carbon atoms in the phenyl groups is only 1.3 A. This is considerably less than a normally bonded carbon-carbon distance of 1.54 A., therefore cis-azobenzene must be

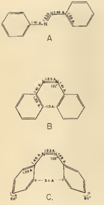
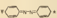


FIG. I CIS- AND TRANS-AZOBENZENE

a nonplanar molecule. The phenyl groups are rotated about the C-N axis and out of the C- = -C plane by an angle of about 50 . In this configuration the ortho carbon atoms are separated by a distance of 3.1 A. This easily accomodates the H atoms. The rotation is accomplished with the expenditure of some energy, for the nonplanar form reduces the resonance energy contribution of important ionic forms such as



These ionic states are important contributors to the planar form of azobenzene. Thus the trans must be the more stable form. The dipole moments of a number of simple benzene compounds have been determined (28). The values are low, and consequently the configuration must be trans as cis compounds have high dipole moments. Thus, trans azobenzene has a zero dipole moment, while the cis isomer has a dipole moment of 3.0 D (141).

Azobenzene is not carcinogenic (143). When injected into rats, aniline, increased ethereal sulfates and glucuronides were isolated (104). The main metabolite was a compound which gave benzidine when it was heated with strong hydrochloric acid. From this it is very probable that the metabolite was hydrazobenzene.

The Millers (208) have tested a large number of azo dyes for carcinogenicity. These are listed in Tables IV and IVa. The same workers (208) have claimed that an interaction takes place between liver protein (s) and the azo carcinogen. In feeding

TABLE IV

RELATIVE CARCINOGENICITY OF AZOBENZENE DERIVATIVES (89,143,206,210,260)

	Compound	Carcinogenic Index	pK_a*
1.	3'-Methyl-4-methylaminoazobenzene	10-12	2.44
2.	3'-Methyl-4-dimethylaminoazobenzene	10-12	2.33
3.	3'-Fluoro-4-dimethylaminoazobenzene	10	-
4.	4'-Fluoro-4-dimethylaminoazobenzene	10	-
5.	2-Fluoro-4-dimethylaminoazobenzene	10-12	-
6.	2',4'-Difluoro-4-dimethylaminoazobenzene	10-12	-
7.	2'-Fluoro-4-dimethylaminoazobenzene	7	-
8.	4-Methylaminoazobenzene	6	2.37
9.	4-Dimethylaminoazobenzene, DAB	6	2.28
10.	4-Methylethylaminoazobenzene	6	2.58
11.	3'-Chloro-4-dimethylaminoazobenzene	5-6	-
12.	2,2'-Dimethyl-4-dimethylaminoazobenzene (286)	4 ?	2.64
13.	2',3-Dimethyl-4-aminoazobenzene	4 ?	2.29
14.	2'-Nitro-4-dimethylaminoazobenzene **	3	-
15.	2'-Methyl-4-dimethylaminoazobenzene	2-3	2.04
16.	2'-Chloro-4-dimethylaminoazobenzene	2	-
17.	4'-Chloro-4-dimethylaminoazobenzene	1-2	2.00
18.	4-Formylmonomethylaminoazobenzene	1-2	-
19.	3'-Ethoxy-4-dimethylaminoazobenzene	1	-
20.	3-Methyl-4-methylaminoazobenzene	1	-
21.	4'-Methyl-4-dimethylaminoazobenzene	0-1	2.36

* See experimental section of paper.
** The 3 isomer has a C.I. of 5.

TABLE IVa

NON-CARCINOGENIC AZOBENZENE DERIVATIVES (89,143,206,210,260)

Compound	pK_a*
22. 4-Hydroxyazobenzene	-
23. 4-Formylaminoazobenzene	-
24. 4-Ethylaminoazobenzene	2.58
25. 4-Diethylaminoazobenzene	3.08
26. 4-Benzylmethylaminoazobenzene	1.57
27. 4-(2'-Hydroxyethyl)methylaminoazobenzene	-
28. 2-Hydroxy-4-dimethylaminoazobenzene	-
29. 2'-Hydroxy-4-dimethylaminoazobenzene	-
30. 3'-Hydroxy-4-dimethylaminoazobenzene	-
31. 4'-Hydroxy-4-dimethylaminoazobenzene	-
32. 2-Methyl-4-dimethylaminoazobenzene	3.08
33. 3-Methyl-4-dimethylaminoazobenzene	-
34. 4'-Nitro-4-dimethylaminoazobenzene	1.80
35. 2'-Trifluoromethyl-4-dimethylaminoazobenzene	-
36. 3'-Trifluoromethyl-4-dimethylaminoazobenzene	-
37. 4'-Trifluoromethyl-4-dimethylaminoazobenzene	-
38. 4-Aminoazobenzene	2.35
39. 2,4-Diaminoazobenzene	4.6
40. 4-Benzalaminoazobenzene	-
41. 4'-Hydroxy-4-aminoazobenzene	-
42. 4'-Hydroxy-4-methylaminoazobenzene	-
43. 3',5'-Dimethyl-4-dimethylaminoazobenzene	-
44. 2',5'-Dimethyl-4-dimethylaminoazobenzene	2.0
45. 2',4'-Dimethyl-4-dimethylaminoazobenzene	-
46. 3,4'-Dimethyl-4-aminoazobenzene	2.39
47. 2',3-Dimethyl-4-monomethylaminoazobenzene	-
48. 2',4',6'-Trichloro-4-dimethylaminoazobenzene	-
49. 2',4',6'-Tribromo-4-dimethylaminoazobenzene	1.0
50. 2',3'-Dichloro-4-dimethylaminoazobenzene	-
51. 2',3',4',5',6'-Pentamethyl-4-aminoazobenzene	-
52. 2,3'-Dimethyl-4-aminoazobenzene	2.92
53. 2,4'-Dimethyl-4-aminoazobenzene	2.92
54. 4-Di-n-propylaminoazobenzene	-
55. 4-Di-n-butylaminoazobenzene	-
56. 4-Di-n-amylaminoazobenzene	-
57. 4-Dimethylaminoazobenzene-4'-sulfonic acid	-
58. 4-Dimethylaminoazobenzene-4'-arsonic acid	-
59. Azobenzene	-
60. 4',5-Dimethyl-2-aminoazobenzene	2.50

* See experimental section of paper.

4-dimethylaminoazobenzene to different animals, these authors found a correlation between the level of protein-bound dye and tumor susceptibility as shown in Table V.

TABLE V

PROTEIN-BOUND DYE AND TUMOR SUSCEPTIBILITY

Species	--M/100mg. Dry Protein-- Bound Azo Dye	Susceptibility
Rat	0.236	high
Mouse	0.070	low
Guinea Pig	0.00	none
Chicken	0.00	none

The Millers also pointed out that in the liver was found the largest concentration of bound dye. In this organ tumor formation took place to the greatest extent. The tumor, once formed, bound less dye than normal tissue. This suggests that the protein which binds the dye is present in normal tissue but is not present in tumor tissue. Electrophoretic studies on the liver protein of rats fed aminoazo dyes seem to bear this out.(277).

In a study of the protein-dye complexes it was found that the more carcinogenic the dye the sooner a maximum concentration of dye-protein complex was reached in the liver (208). It is now known that in the body butter yellow is demethylated, bound in an unknown manner to some protein, reductively cleaved and probably oxidized in the extended para position. This last has not been definitely proven, but has been shown *in vitro* (218).

II

BACKGROUND OF THE PROBLEM

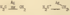
A. Acids and Bases

1. Introduction. All the aromatic carcinogens are electron donors. Therefore they are bases in the Lewis sense. Specifically the amine carcinogens are hetero bases, while both the amine and the polynuclear carcinogens are pi bases. (Hetero and pi basicity will be discussed more fully in the section on bases). Because of the universal electron donor properties of the aromatic carcinogens a thorough study of basicity and acidity is necessary towards a proper understanding of the carcinogens.

In an important paper on acids and bases Lewis (185) defined an acid as a substance which could accept an electron pair, a base as a substance which could donate an electron pair. This concept has been further developed by Mulliken (220) in a quantum-mechanical study of the molecular compounds formed by interaction of electron donors and acceptors.

2. Acids or electron acceptors. Mulliken has subdivided the acids into (a) ionic, or i, acids (b) dissociation, or d, acids, (c) vacant orbital, or v, acceptors, and (d) pi acceptors.

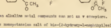
a. Ionic acids. Some examples of i acids are O, Ag^+, Na^+, H^+, etc. The i acids can combine with all three types of bases, e.g., ionic, hetero or pi bases. An example of an i acid reacting with an i base is $H^+ + {:}OH^- \rightarrow H_2O$. An example of an i acid reacting with a [illegible] $:CH_2 \rightarrow RNH_3^+$. For an acid to combine with a pi base, the i acid must have an appropriate size so that it will be able to react with the fairly large pi system. For example Ag^+ and ethylene are of a comparable size. They have been found to form an addition compound (301). It is probable that the following type of complex is formed,



where the atom is centered over the plane of the ethylene molecule. The formation of a complex between the silver ion and benzene derivatives has been confirmed through solubility and absorption spectra studies (8,9,10,167). In the $Bz \cdot Ag^+$ complexes the silver atom is believed to be above and between two carbon atoms of the ring (220), as in ethylene. The complexes are more stable the greater the basicity of the pi base, except when there are too many substituents on the ring and then steric hindrance interferes with the approachability of the Ag^+ (220). The study of the reaction of the stronger pi bases such as base (a) anthracene, chrysene, etc., with Ag^+ have not been undertaken as yet. The reaction of the amine pi bases with Ag^+ has not been studied, so far as the author knows, although o-phenylene-diamine forms a crystalline complex with silver nitrate (299). These studies would be of great theoretical interest.

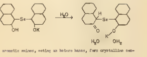
b. Dissociation acids. Some examples of this type of acid are HX, X_2 and RX where X stands for a halogen atom. The interaction of d acids such as ICl (166), I_2 (25,150) and Br_2(165) with benzene and its derivatives to form 1:1 complexes has been discussed by Mulliken (219,220). The halogen – aromatic complexes and the silver ion – aromatic complexes give an intense characteristic electronic absorption spectra which is said to be caused by charge-transfer forces which arise from intermolecular interaction. The stability of the known halogen – benzene derivative complexes increases with an increase of the basicity of the parent pi base. An investigation of the naphthalene complexes with iodine and bromine has disclosed (35) that these complexes are stronger than the analogous benzene complexes. This is not surprising as naphthalene is a stronger pi base than benzene. It would be of interest to study the reaction of the stronger pi bases, such as the polynuclear carcinogens and the carcinogenic amines, with d acids.

e. Vacant orbital, or v, acceptors. Some examples of this type of electron acceptor are BR_3, AlX_3 and SnX_4. The v acceptors combine especially with n bases to form strong complexes, e.g., (265),



Even alkaline metal compounds can act as v acceptors. For example; the monopotassium salt of bis-(2-hydroxy-1-naphthyl)selenide, in the presence of water, changes into a yellow chelate compound, melting at

170° and soluble in ether, toluene and chloroform (102).



The aromatic amines, acting as hetero bases, form crystalline complexes with the tin halides. In this respect the molecular compounds formed by carcinogenic amines and v acceptors might be worthy of study. For compounds of this type the relative binding strength would be greater the lower the ionization potential of the carcinogenic amine, the greater the electron affinity of the acid and the smaller the interference to the convergence of the two parent compounds.

The possibility of a Lewis base-acid reaction between a pi base and a v acceptor has not been thoroughly studied, although molecular complexes are known of the composition $(CH_3)_{16}Pt_4 \cdot 2C_6H_6$ (239) and $Ni(CN)_2 \cdot NH_3 \cdot M$ (242) where M is benzene, thiophene, furan, pyrrole, aniline or phenol. The consensus of opinion at the moment is that these complexes are clathrate molecular compounds where 1 mole of the aromatic compound is imprisoned in a cage structure of the other components of the compound.

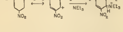
d. Pi acceptors. Examples of some fairly strong pi acids are trinitrotoluene, s-trinitrobenzene (65,282), picric acid (288), 2,4,7-trinitrofluorenone (225,226), chloranil (189,205,232), 2,2',4,4'-tetranitrobiphenyl, 2,7-dinitroanthraquinone, and 5-nitro-1,3-benzene disulfonyl chloride (26). The acidic strength of these acids is directly proportional to the number and type of electronegative groups and inversely propotional to the number and type of electropositive groups attached to the aromatic hydrocarbon. In the formation of a pi complex the size, shape and constitution of both the pi acid and the pi base are of great importance. The geometry and theory of ion complexes will be considered in the section devoted to pi bases.

In the pi acceptors the electronegative groups pull electrons out of ion ring, thus causing a deficiency of electrons in the ring. On the other hand in the pi bases, such as naphthalene and anthracene, there is an 'excess' of electrons in the rings. It is apparent that the presence of electron-repelling groups, such as NH_2, OCH_3, etc., on the ring will force more electrons into ion ring. Thus conditions are ripe for the pi system of these two diverse types of molecules to interact. The consequent pi-electronic transitions in the third dimension (perpendicular to the planes of the two parent pi systems) result in light absorption further out in the visible because of the formation of a comparatively low energy Z band (see 92). This is the explanation for the deeper color of the complex as compared to the starting products. On this basis one would expect weak pi acids and bases to interact to form unstable light colored complexes. In this respect when pure aniline and pure nitrobenzene are mixed, an orange color is formed. This is attributed to a Lewis acid-base reaction (123). The

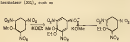
Same authors also pointed out that when a weaker base such as phenethylamine and nitrobenzene were mixed, no color was formed. Through a study of the absorption spectra it has been shown that the weak pi base, aniline, forms 1:1 complexes with m-dinitrobenzene, p-dinitrobenzene and s-trinitrobenzene (182). The conclusions of the authors are that the weak intermolecular binding in the complexes is the result of resonance between a no-bond structure and a charge transfer, or ionic, structure. The s-trinitrobenzene complex has the greatest stability as would be expected, for this latter complex has been isolated (147).

When the strength of the pi base is increased slightly, crystalline complexes can be isolated. For example, the weak complexes, m-dinitrobenzene-naphthalene, m.p. 52-53, and p-dinitrobenzene-naphthalene, m.p. 118-119, are known (148). The makeup of the pi base will determine in what proportions a reference acid and the base will interact or whether they will interact at all. For example, 9-cinnamalfluorene combines with 2 moles of picric acid, 9-benzalfluorene with 1 mole picric acid, while 9-benzylfluorene does not form a complex (288). Compounds with two separated ring systems, conjugated or non-conjugated, tend to combine with two moles of s-trinitrobenzene, for example - - diphenylamine and benzalaniline (282), 4-dimethylaminoazobenzene, 2,2'-diaminodiphenyl disulfide, dibenzyl, stilbene, diphenylacetylene, 1,1'-azonaphthalene, 4-aminodiphenylamine, and 4-aminodiphenylmethane (283).

Meta substituted pi acids also react with hetero bases, but in this case they act as v acceptors. For example 1,3,5-trinitrobenzene is neutralized by ammonia, methylamine, dimethylamine or triethylamine to give a violet colored quinonoidal compound (187), e.g.,



Meta substituted pi acceptors can also react with i bases, such as $(CH_2-CO-R)^-$, $(OEt)^-$ and $(NH_2)^-$. Here again the pi acids act somewhat as v acceptors in that a weak, unstable bond is formed between the pi acid and the anion. The quinonoid complex has a purple to violet color. The structures of these complexes are based on the extensive work of Canback (68,69,70,72,73,74) and on the synthetic reactions of Meisenheimer (201), such as



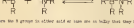
The formation of a violet to blue color by the reaction of m-dinitrobenzenes with liquid ammonia (112,121) and with primary and secondary aliphatic amines (72) is most likely caused by the formation of a similar quinonoid anion. New developments in this field are presented in Part V of this paper. Peculiarly enough the sex hormones, which contain keto groups in the 3 or 17-position, give a color reaction with alkaline m-dinitrobenzene, while physiologically inactive steroids, which have their keto groups in the 6, 7 or 12-position, do not give the color reaction (309).

3. Bases or electron donors. Mulliken (220) has subdivided the electron donors into (a) ionic, or i, bases (b) hetero, onium, or n, bases, and (c) pi bases.

a. Ionic bases. Some examples are OH^-, OEt^-, NH_2^- and CN^-. The reactions of i bases have been discussed in previous sections of the paper.

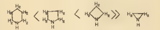
b. Hetero bases. As the carcinogenic amines are n bases (approximate pK_a in water of 3.0 to 4.5) the properties of n bases are of particular interest to the cancer chemist. In the reaction between an n base and an acid the formation of a strong stable complex is favored by the low ionization potential of the base, the high electron affinity of the acid and advantageous steric conditions. The steric effects encountered in acid-base reactions have been investigated by Brown *et al.* (48,49,50,51,52,53,54). The basic strengths of ortho substituted aromatic amines are dependent on (a) inductive and resonance effects of groups substituted on the nitrogen and in the ring, (b) the amount of interaction between the free electrons on the nitrogen and the pi electrons of the hydrocarbon, (c) steric inhibition of resonance in the n base between the amine group and the ring, and (d) the increase in steric strain on the addition of a proton or a v acid to the base (because of the transformation of a N atom from its trivalent configuration in the free base to the bulkier tetrahedral configuration in the onium ion) (53). Other things being equal the greater the steric strain of the onium ion, the greater the decrease in basicity. In the aromatic amines steric effects can be made so great that cis and trans forms can be isolated. For example, cis and trans derivatives of N,N'-diisopropyldibenzenesulfonamidomesitylene have been isolated (2).

Brown has introduced the concept of F, B and I strains in acid-base reactions. Frontal, or F strain is illustrated by



where the R groups in either acid or base are so bulky that they interfere with the approachability of the two groups. Back, or B strain arises when an n base combines with an acid. The tendency to form tetrahedral angles meets opposition from repulsive forces between the groups on the N at the back of the molecule. The magnitude of the B strain would depend on the number, nature and size of the substituent groups on the nitrogen. I strain has been defined (52) as that change in internal strain of a ring compound which results from a change in the coordination number (and the preferred bond angle) of a ring atom involved in a reaction. Using ethylenimine as an example Brown states that this molecule contains a strain involved in bending the two N–C bonds from their natural value of 90° to the 60° required by the geometry of the ring. On the other hand the ethylenimine-boron trimethide molecule may be considered to contain the larger strain involved in bending this angle from the 109° 28' value of a tetrahedral coordinated nitrogen back to the approximately 60° value fixed by the ring. The effect of I strain and F strain is shown on the stabilities of boron trimethide complexes of the following heterocyclic compounds (52).

F strain decreases



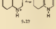
I strain increases

Ignoring steric effects, it is very probable that the base strength decreases in each of the following series $NR_3 > PR_3 > AsR_3 > SbR_3$; $OR_2 > SeR_2 > TeR_2$; $NR_3 > OR_2 > ClR$ (220).

As we are dealing with the carcinogenic amines, which are aromatic, we have another important property of the molecule which will affect the basicity. This is the n → pi transition (see IIIB3 for a discussion). Ignoring steric and sigma electron effects the n electrons on the N of an aliphatic amine are wholly available for reaction with an acid, but in the aromatic amines a new factor has been added. There is an interaction between the free electrons on the N and the pi electrons of the aromatic hydrocarbon. Consequently we have a fairly strong n → pi transition. The electrons on the N are not as available to acids, so there is a loss in basicity. This effect is shown very strongly by heterocyclic compounds. Pyrrole, in which there is a practically complete n → pi transition of the free electrons on the N, is an extremely weak base with a pKa of approximately 0.4 (137). Pyridine is a much weaker base than piperidine because of the partial n → pi transition of the former.

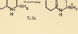
Albert and Goldacre (3,4,5) have introduced the concept of the ionic resonance effect in their papers on the dissociation constants of heterocyclic amines. Essentially this effect is due to a simultaneous n → pi, pi' → n' transition involving 2 N atoms, which we will call a pi n' ↔ n pi' transition for the sake of brevity. In the monoamine derivatives of pyridine, quinoline and acridine, the compounds with the amino group para to the ring nitrogen are the most basic (5). This is due to the base strengthening pi n' ↔ n pi' transition.

pKa 4.94

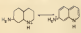


9.17

The effect is still powerful in the ortho amino compounds such as



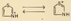
7.34



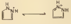
6.65

In the remainder of the amines of pyridine, quinoline and acridine the basicity is much weaker.

When another nitrogen is inserted into a five membered ring, the effect is base-strengthening. For example, pyrazole, glyoxaline, benzimidazole and indazole are stronger bases than the iso-pi-electronic mononitrogen compounds (5). This is caused by the pi n'↔n pi' transition, e.g.,



pKa 2.5

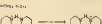


pKa 2.53

When there are several possibly basic nitrogens in the ring of a molecule and the pi n'↔n pi' transition can only take place with difficulty, there is an interaction between the free electrons on the nitrogens. This interaction causes base-weakening n electronic transitions (see XIIB). Apparently in this type of interaction the free electrons on the nitrogen atoms are not as available for salt formation. The effect is very striking in the pyridine series (5).

pKa 5.23 2.33 1.30 0.6

One would expect pyridazine to be a much weaker base, but in the salt a pi n' ⟷ n pi' transition could probably take place due to hydrogen bonding, e.g.,



This would cause some base-strengthening effect. Further evidence for the n electronic transitions in these three weakly basic molecules is the presence of n bands (see IIIB2) in their absorption spectra. Even saturated molecules show this base-weakening effect to some extent. For example, hydrazine, pKa 7.94 (308), is a weaker base than ammonia, pKa 9.3, while piperazine, pKa 10.3 (176), is weaker than piperidine, pKa 11.6.

The base-weakening effect of halogen atoms has been shown. The order of decreasing basicity in o-, m- and p- substituted aniline derivatives (27) and in p- substituted dimethylaniline derivatives (93) is H > F > Cl > Br > I. In the halogen substituted aniline derivatives the order of decreasing basicity for the different positions is p>m>o. The ortho derivatives are least basic because of the base-weakening properties of the ortho effect. This effect is mainly comprised of inductive, resonance and steric factors. For example (179), strong inductive and resonance effects and the steric strain of the ion weakens the basicity of o-nitroaniline to a pKa of -0.3. In m-nitroaniline only the inductive effect tends to weaken the basicity to a pKa of 2.50, while in p-nitroaniline a very weak inductive effect and a strong resonance effect reduce the basicity to a pKa of 1.0 (aniline, pKa 4.58). To show the complexity of the ortho effect we will consider dimethylaniline, pKa 4.26, dimethyl-o-toluidine, pKa 5.07, and dimethyl-m-2-xylidine, pKa 4.49 (289). The introduction of an o-methyl group has increased the basicity due to the fact that the methyl group has pushed the dimethylamino group out of the plane of the molecule, thus causing a base strengthening partial pi→π transition. But the formation of a salt involves steric strain between the $-\overset{+}{N}H(CH_3)_2$ and the $-CH_3$. As the steric strain is not large this causes a slight base weakening effect. The overall effect is base strengthening. On the other hand the introduction of two o-methyl groups causes a larger pi→π transition but at the same time the steric strain becomes much greater. Consequently there is a base weakening effect as compared to dimethyl-o-toluidine.

e. Pi bases. The carcinogenic amines and the polynuclear carcinogens are pi bases. For that reason the physical and chemical properties of this type of base are of great interest to the cancer chemist. A pi base is essentially an unsaturated molecule which is a source of pi electrons. It is very probable that the polynuclear and amine carcinogens are very strong pi bases with approximately the same order of basicity. This line of reasoning is based mainly on the qualitative results in pi base-pi acid interactions, for quantitative work in this field is very recent and superficial.

Many of the reactions of pi bases have been discussed in the section of the paper devoted to acids, but the most typical reaction is that with a pi acceptor. In this type of molecular compound the influence of the pi base on the color of the complex is what one would expect. The presence of electronegative groups lightens the color while an increase in conjugation or the presence of electropositive groups causes a bathochromic effect. This is shown in Table Va where the molecular compounds combine with s-trinitrobenzene in the usual 1:1 mole ratio, except for fluorene-s-trinitrobenzene (2:3) and 2-aminofluorene-s-trinitrobenzene (3:4).

Certain types of 'flaws' in the pi system of a pi acid or a pi base which tend to weaken pi electronic interactions in the molecule have a powerful effect on the stability of the complex. Some of the 'flaws' are the presence of groups which can function as fairly strong i acids or fairly strong hetero bases. For example, p-sulfanilic acid and o-hydroxyaniline do not form complexes, while ethyl p-sulfanilate and o-anisidine do form complexes with s-trinitrobenzene (282).

As the pi acids and bases interact face to face (239,240,241), their approachability is at a maximum when the two molecules are

TABLE Va

COLOR OF s-TRINITROBENZENE ADDITION COMPOUNDS (65,282,283)

Pi base	Color
Naphthalene	pale yellow
1-Nitronaphthalene	pale yellow
1-Bromonaphthalene	lemon yellow
Dibenzofuran	lemon yellow
Fluorene	gold yellow
1-Nitro-2-naphthylamine	gold yellow
1-Naphthylamine	deep red
Anthracene	scarlet
1-Naphthol	red-brown
1-Ethylanthracene	scarlet
2-Aminofluorene	red-chocolate
1,2-Diaminonaphthalene	purple
2,7-Diaminofluorene	black
2-Anthrylamine	black

planar. Thus non-planarity of either acid or base can interfere with complex formation. For example, diphenylamine forms a complex while N,N'-diphenylacetamide does not form a complex with trinitrobenzene (282). On the assumption that "the melting point of a complex can generally be used as a criterion of the stability of the complex", Orchin (228) has pointed out that in the monomethyl benz(a)anthracene 'picrates' the 1- and 12- methylbenzanthracene complexes have the lowest melting points. Both of these compounds involve substitution at a position where steric interference between a methyl group and a hydrogen is possible. Interestingly enough 1-methylbenz(a)anthracene is the only monomethylbenzanthracene which forms a complex with two moles of picric acid. This is believed due to its lack of planarity, which causes the molecule to function as two naphthalene nuclei each of which is capable of reacting with a picric acid molecule. The same explanation is given for the fact that 7,12-benz(a)anthracene is the only known dimethylbenzanthracene which forms a dipicrate (228). Orchin has also pointed out that trans-stilbene and 2-phenylnaphthalene form picrates while the non-planar cis-stilbene, 1-phenylnaphthalene and 2-o-tolylnaphthalene do not. It may be pointed out that the geometrical makeup of both the acid and the base is of great importance in determining the stability of the complex. In this respect it will be noted that in the series benzene, naphthalene, anthracene and naphthacene, benzene forms a very unstable complex and naphthacene (149) does not form a molecular complex with s-trinitrobenzene while naphthalene and anthracene do so readily. For this reason the relative basicity will vary, dependent on the reference acid used.

It has been shown (182,221) that in the pi complexes the intramolecular distances between the planes of acid and base are approximately 3.2 A. which is considerably shorter than the usual distance between molecules. The acid and base molecules are packed close together in the crystal but do not have a coaxial configuration. For example, in the 1:1 crystal complex of p-iodaniline and s-trinitrobenzene the shortest intermolecular distance is between the nitrogen atom of an amine group and its oxygen neighbors in the nitro group (240). Resonance and color have been based on two dimensional chemistry, but it is apparent from absorption spectra studies that pi electronic interactions can still take place between two halves of a conjugated pi system even when they are not coplanar. This idea has been thoroughly discussed in a recent paper on biphenyl derivatives (21). On the other hand in the pi complexes (93) pi electronic interactions take place in the Z direction, i.e., perpendicular to the planes of the parent molecules. The concepts of electron transfer (296) and charge transfer (220) are in line with this reasoning. From the measurements of dichroism in molecular compounds Nakamoto (221) has reached the same conclusion that "The nature of the intermolecular force in the pi-pi attraction is the perpendicular direction by the approach of both components due to mutual polarizing forces".

B. Absorption Spectra

1. Outer electrons. To properly understand the aromatic carcinogens we must first study and understand their outer electrons. To a large extent it is the outer electrons which determine the chemical and physical properties of a molecule. More specifically the pi and n electrons are the major factor in the chemical activity and the absorption spectra (2000-8000 A.) of the aromatic molecule. For this reason the absorption spectra of a large number of carcinogenic amines and allied compounds have been studied in this paper. In the amine and the polynuclear carcinogens, as in all conjugated organic molecules, there are two groups of outer electrons which determine the spectra in the ultraviolet and the visible regions. These are the delocalized, or pi electrons, in conjugated molecules and the nonbonding, or n electrons, on hetero atoms. There is also a third group of outer electrons called the bonding, or sigma, electrons, which are responsible for the basic framework of normal single bonds. These electrons are illustrated in the structural formula of dibenzofuran shown below.

• n electron

— 2 sigma electrons

x pi electron

Absorption of light of wave length 2000 to 8000 A. corresponds to energy differences of between 143 and 36 kg. cal./mole. Energy increments of this magnitude are sufficient to allow the displacement of pi and n electrons of aromatic carcinogens. The energy available in the vacuum ultraviolet displaces sigma electrons, thus causing photoionization of normal single bonds while the energy available in the infrared is just enough to cause vibrational and rotational transitions.

The properties of pi electrons in conjugated molecules, such as the aromatic carcinogens, can be pictured as follows. (a) Each pi electron is described by an orbital and this orbital extends over the conjugated part of the molecule. (b) Each orbital has energy associated with it. The approximate total energy of the pi electrons is found by simple summation of the energies of the electrons over the occupied orbitals. (c) In determining the configuration of the pi electrons each orbital is expressed as a linear combination of atomic orbitals of the correct symmetry (97). In this approximation (LCAO approximation) the total number of pi molecular orbitals in a hydrocarbon equals the number of pi electrons, since both are equal to the number of available 2p atomic orbitals.

2. Spectral bands. Two main types of bands important in a study of the spectra are (a) n electronic transition bands and (b) pi electronic transition bands.

When the n bands of hetero groups are attached to pi systems of a given size, they move to the red in the approximate order $-COOH$, $-C=N-$, $-N=C-C=N-$, $-NO_2$, $-C\equiv N$, $-C=O$, $-N=N-$, $-COCO-$, $-NO$ (236). The n bands are generally weaker in intensity than the pi bands because the n orbitals are local, while the pi orbitals are spread over the whole conjugated system. The n bands have molecular extinction coefficients between 10 and 100, while the extinction coefficients of the pi bands run from 200 to over 30000. Increasing the size of a conjugated system adjacent to a hetero group usually causes a much greater bathochromic effect on the longest wave length pi bands than on the n bands (59). The effect of an auxochromic group in direct contact or in a para position to a hetero group is to move the longest wave length pi electronic transitions further into the visible while the n band is moved in the opposite direction or concealed by the pi band. This reverse effect on the n band has been shown in the series - - thiobenzophenone, 4,4'-dimethoxythiobenzophenone and 4,4'-bisdimethylaminothiobenzophenone. The n band maxima for these compounds are 6050, 5925 and 5730 A. respectively (59).

In the directional pi band the extent of interaction between a molecule and a beam of light is determined not only by the properties of the light and the molecule, but also by the orientation of the molecule with respect to the electric vector of the light wave. In the case of a non-symmetrical planar molecule, such vector effects would give rise to 2 bands resulting from the 2 electronic activation processes associated with electric moments oriented normally to each other in the plane of the molecule (186). Absorption bands resulting from the primary activation process of lower energy are called X bands and those of higher energy Y bands. Examples of compounds with X and Y bands have been given in the crystal violet series (110). In certain cases there will even be molecules with an appreciable electronic interaction in three directions whose spectra will have 3 maxima. The new band is called a Z band. It is due to a face-to-face 'polymerization' wherein an intermolecular electronic interaction takes place (110).

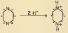
Another type of pi band is the X' band resulting from a higher excitation to a second excited state. When an aromatic hydrocarbon containing an electropositive group is substituted in the para position with an electronegative group a new spectral band, an X' band, is usually formed. Para substituted phenol or aniline derivatives show this phenomenon (100,181).

Separate absorption bands may arise from insulated resonating systems. The two separate conjugated systems give rise to two separate excited states and, consequently, to two bands. These bands are called partials (216).

3. Electronic transitions. An important tool that will prove of great value is a study of the spectral bands of the aromatic carcinogens is the electronic transition.

The low energy shoulder observed in the absorption spectrum of pyridine in cyclohexane is probably due to the n electrons on the nitrogen. In water solution this shoulder disappears. It is believed that the shoulder arises from a nonbonding electron transition with water exerting a bonding effect on the n electrons of the nitrogen shifting the transition to higher energies (139). This is an example of an n->sigma transition. When 2 or more hetero atoms with n electrons are in proximity in a molecule, their n orbitals will interact to produce double or multiple n-bands and the n-bands will be moved into a region of lower energy (i.e., further into the visible). The diazines - - pyrazine, pyrimidine and pyridazine - - show this effect, for the low intensity longest wave length bands of these molecules are n bands which are much further in the visible than the pyridine shoulder (139).

The n bands of pyridazine and pyrazine move toward the ultraviolet and lose their fine structure when water replaces cyclohexane as a solvent. Here again, water probably effects a weak bonding effect on the n electrons of one nitrogen causing an n→sigma transition. Similarly the monomethiodide salts of these diazines show the n→sigma transition. Both pairs of n electrons in pyrazine should be bound in concentrated sulfuric acid solution. Consequently the n band should be obliterated. This was shown to be so (139). The reaction would be



The nitrogen on an amine group has 2 n electrons. When this group is attached to an aromatic molecule, the non-bonding electrons on the nitrogen interact with the pi electrons of the conjugated system and become part of the pi electron system. Thus we have an n→pi transition. As a first approximation we could say that the degree of conjugation of an amine group or, for that matter, any hetero group with a conjugated system depends on (a) the strength of binding of the free electrons to the hetero atom, (b) the overlap or interaction of these free electrons with the pi electrons of the conjugated system and (c) whether the connection to the parent pi system is single (as in 2-aminofluorene) or multiple (as in carbazole).

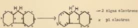
Many examples of the pi → n transition can be cited among the aromatic amines. In dimethylaniline the free electrons on the nitrogen of the dimethylamino group can interact freely with the pi electrons of the benzene ring and as these electrons are pi electrons. When a t-butyl group is placed in an ortho position to the dimethylamino group, the latter is pushed out of the plane of the molecule. Consequently the free electrons on the hetero group can not interact so freely with the pi system. As a result there is a pi → n transition and the new molecule resembles an ortho dialkylbenzene much more than it resembles dimethylaniline, especially in respect to absorption spectra and chemical properties (230).

When a proton becomes attached to the free electrons on the nitrogen of an aromatic amine, we have a pi → sigma transition. This is shown by the fact that the absorption spectra of the salt resembles the spectra of the aromatic hydrocarbon much more than it resembles the spectra of the aromatic amine.

Ionization and probably free radical formation are examples of the sigma → n transition, e.g., $H-Cl \rightarrow H^{+} + :Cl^{-}$

$$R_3C-CR_3 \rightarrow 2R_3C\cdot$$

Hyperconjugation is an example of a weak sigma →pi transition, i.e.,



Here the 'ionization' of the hydrogen has converted its sigma electrons into pi electrons with the conferring of partial double bond character upon every carbon to carbon bond.

4. Spectral effects. a. Fine structure, or Fs effect. In non-rigid molecules, such as diphenylmethane, biphenyl and 2-hydroxybiphenyl, the excitational energy absorbed in the ultraviolet can cause group vibrations or rotations. On the other hand in comparatively rigid molecules like fluorene or dibenzofuran the absorbed energy cannot be readily dissipated as rotational energy but manifests itself as electronic vibrational energy. Consequently diphenylmethane and biphenyl have much less fine structure than fluorene and 2-hydroxybiphenyl has less fine structure than dibenzofuran (Fig. 40). As hydrindene is a more rigid molecule than o-xylene, one would expect the former molecule to have more fine structure. This has been shown to be so (246). In the compounds dibenzyl, stilbene and diphenylacetylene the rigidity of the molecule increases as the unsaturation increases. Consequently diphenylacetylene (267) has a greater Fs effect than stilbene (32), while stilbene has a greater Fs effect than dibenzyl (188).

Consider a solute in solution. The lower the temperature of the solution, the more 'rigid' the solvent and the greater the decrease in movement of the parts of a solute on absorption of energy. As a result the solute becomes more rigid at a lower temperature and absorbed light is converted to a larger extent into electronic vibrational energy. Thus, there is an increase in fine structure on lowering the temperature (274).

In the case of the diphenyl polyacetylenes the lengthening of the linear chain would present a longer 'rigid' conjugated molecule over which there would be a greater amount of electronic vibrational interplay. Consequently as the number of acetylene groups are increased, the Fa effect would become stronger. This has been shown to be so (267).

The polarity of a solvent can have a large affect on the fine structure in the spectra of a molecule. This is mainly due to hydrogen bond formation and a consequent n→sigma or pi→sigma transition. For example, phenol in alcohol shows a definite -Fa affect as compared with phenol in isooctane (82).

The affect of a halogen atom on the spectra of a compound has been discussed by Ferguson (110). He contends that the larger halogen atom tends to absorb more light energy, consequently less energy is available for electronic vibrational energy. As a result fine structure decreases in the following series as the substituent increases in size - - benzene, fluorobenzene, chlorobenzene, bromobenzene and iodobenzene.

Many other examples of the Fe effect have been given by Kortum and Dreesen (178), but the facts presented are sufficient to show the trends in this effect.

b. Bathochromic, or B, effect. When a molecule is in the path of radiation of the proper wave length there is an induced oscillation of electrons throughout the molecule. The absorption of light causes the transition of the molecule from its ground state to an ionic electronically excited state and the difference of energy of these two states will determine the frequency of the light absorbed. The smaller the difference the further into the visible will a molecule absorb, i.e., the greater will be the B effect. The B effect will be larger the greater the resonance among ionic forms or forms involving a separation of charge, the longer the conjugation in a molecule, and the greater the strain in a molecule.

c. Steric, or S, effect. Groups which are attached to aromatic hydrocarbons are influenced in an anomalous fashion by ortho substituents. This is called the ortho effect. Some of the factors that comprise this effect are steric hindrance, steric strain, chelation, the field effect through space and the inductive effect. Remington (250) has presented the curves of a large number of dimethylaniline derivatives. When a methyl, chloro or bromo group was substituted in the ortho position to the dimethylamino group, the intensity was decreased considerably. Substitution of 2 methyl groups in the ortho positions decreased the intensity of absorption to an even greater extent, while the substitution of the t-butyl group decreased the intensity down to the benzene level. Klevens and Platt (171) report that in the N,N-dimethylanilines the size of the ortho group is related to the angle of twist of the $-N(CH_3)_2$ group with the plane of the molecule. With a 90° angle of twist the spectrum becomes almost identical with that of a simple alkylbenzene. Groups do not necessarily have to be in the ortho position for steric hindrance to take place. In the case of stilbene and azobenzene the band furthest out in the visible has a much smaller intensity for the cis derivatives than for the trans (214). This decrease in intensity is evidently due to steric hindrance.

d. Intensity, or I, effect. Ferguson (110) has stated that the intensity of an absorption band depends partly upon the probability of the transition from the ground to the ionically excited states of the molecule, upon the polarity of the excited states, and upon the magnitude of the electronic displacements involved in passing from the normal to the excited states. An example of an increasing intensity effect is seen in the series - - isopropylbenzene, cyclohexylbenzene, cyclopentylbenzene, cyclopropylbenzene and styrene.

5. Types of sequences. In a study of the absorption spectra of a compound an important tool in elucidating what different parts of the curve mean is the sequence. There are six main types - - isohetero, variconjugate, homologue, substitution, position and solvent.

In the isohetero sequence the hetero group is kept constant while the remainder of the molecule is changed. The effect of a hetero-group on the spectra of a compound could thus be studied. The marked conjugating a [illegible] sulfone group directly attached to a benzene ring has been proven by a study of the sequence - - benzenesulfonamide, cyclohexylphenylsulfone, diphenylsulfone and dibenzylsulfone (174). From a study of the spectra of a large number of sulfones it has been concluded that the sulfonyl sulfur atom is capable of accommodating, at least temporarily, more than eight electrons in its valence shell (108).

In the varioconjugate sequence the conjugated group is kept constant while the hetero group is gradually changed in such a way that the pi electrons on the hetero group interact to an increasing extent with the parent pi system. Many examples of this type of sequence have been given by Platt in his excellent paper on the varioconjugate sequence (236). Platt states that any two organic molecules in such a sequence are iso-pi-electronic if they have the same number of pi electrons, with approximately the same geometrical arrangement and bound about equally tightly, in a planar conjugated system. Thus dibenzoselenophene and phenanthrene are iso-pi-electronic. An important example of the varioconjugate sequence is the series - - fluorene, dibenzofuran, dibenzothiophene, dibenzoselenophene, carbazole and phenanthrene. This particular example is a K region sequence, which will be more fully discussed in Part VIA.

The homologue sequence is of value in a study of some member (s) of a homologous series. Clar (79) has used this sequence in extensive studies of the polynuclear hydrocarbons.

A substitution sequence is essentially a study of the effect of a large number of substituents on the absorption spectra of a molecule. This sequence is of value in a study of spectral effects and spectral bands. Using this type of sequence Kumler (181) substantiated the existence of X' bands in the spectra of eighteen compounds of the type

where A is an electron-donor group and B is an electron-acceptor group.

A position sequence is a comparison of the spectra of position isomers. For example, the aminophenanthrene sequence would include the spectra of the 1, 2, 3, 4 and 9-aminophenanthrenes. Such a sequence would give no information about the excited states of the parent compound. In the example given above, the sequence would show what influence the Fe effect of the K region and the -Fe and B effects of the diverse amino groups would have on the comparatively low energy, low intensity, vibrational bands of phenanthrene. Jones has used this sequence in a correlation of the carcinogenicity of the methylbenzanthracenes versus the absorption spectra (158).

A solvent sequence is a comparison of the spectra of a compound in various solvents. One example is the examination of the effect of changing pH upon the absorption spectra of a compound (139). Sheppard and Newsome (273) have investigated the effect of the dipole moment of the solvent upon the spectra of 4'-nitro-4-dimethylaminoazobenzene. They investigated over thirty solvents. Halverson and Hirt (139) have concluded, with the help of the solvent sequence, that the low energy band systems of pyrazine and pyridine are due to n-electronic transitions while the high intensity band systems are allowed pi-electronic transitions. Bayliss (20) has developed an equation which relates the B effect due to the solvent (ΔV) with the oscillator strength (f), the size of the solute molecule, and the index of refraction. Thus

$$\Delta V = K \left(\frac{f}{Va^3}\right) \left(\frac{n^2 - 1}{2n^2 + 1}\right)$$

where a is the radius of the solute molecule, and n is the refractive index of the solvent at the wave length of the absorption band. On the basis of this equation one would expect a linear relation between ΔV and $(n^2-1)/(2n^2+1)$. Badger (16) found in a study of the spectra of rubrene in nineteen solvents that an approximate linear relation did exist between ΔV and $(n^2-1)/(2n^2+1)$ when diethyl ether was used as the reference solvent.

IV

EXPERIMENTAL

A. Syntheses

o-Phenylenediamine. The Eastman Kodak Co. practical grade product was crystallized from hexane, m.p. 103-104° . Lit. 102-103° (257).

1,2-Diacetylaminobenzene was formed by the reaction between a saturated benzene solution of o-phenylenediamine and slightly more than 2 equivalents of acetic anhydride for one hour at room temperature. Two crystallizations from water gave colorless crystals, m.p. 185-186° (34).

p-Nitrobenzyl chloride was formed by treatment of benzyl chloride with a mixture of fuming nitric acid and sulfuric acid. The separated solid was crystallized twice from heptane in long colorless needles, m.p. 71-72° . Lit. 71° (7).

2-Carbethoxyaminonitrobenzene was obtained in practically quantitative yield by the following procedure. To an ice-cold solution of nitroaniline in pyridine was added dropwise an equivalent of ethyl chlorocarbonate. Half an hour after the addition, the mixture was poured into excess dilute hydrochloric acid. Yellow needles were obtained from pentane, m.p. 56-57° . Lit. 58° (257).

Benzo(2,1,3)selenadiazole, also called piaselenole, was prepared by refluxing o-phenylenediamine with an equimolar amount of selenium dioxide in alcohol for 0.5 hour. Light yellow needles were obtained from hexane, m.p. 75-76° . Lit. 76° (151).

Benzimidazole was prepared by refluxing o-phenylenediamine in excess formic acid for 0.5 hour. Crystallisation from hexane gave colorless crystals, m.p. 171-172° . Lit. 167° (306).

p-Dimethylaminobenzalaniline. One equivalent of aniline and 1 equivalent of p-dimethylaminobenzaldehyde were heated for 0.5 hour at 100° . The product was crystallized from hexane and then from aqueous alcohol, m.p. 101° . Lit. 100° (261).

9,10-Dihydroacridine. Acridine was reduced by zinc dust and hydrochloric acid. Two crystallisations from heptane gave colorless needles, m.p. 169° . Lit. 168° (30).

Xanthone was prepared by the reaction of salicylic acid, phenol and acetic anhydride (96). Colorless needles were obtained from alcohol, m.p. 174° . Lit. 173-174° (155).

Anthrone was obtained by the reduction of anthraquinone in acetic acid with zinc dust and hydrochloric acid. Colorless needles from benzene-pentane melted at 154-155° . Lit 154-155° (204).

Azobenzene was prepared by the reduction of nitrobenzene with sodium stannite. Orange crystals were formed from pentane, m.p. 67-68° (303).

1,3-Diphenyltriazene was synthesized by coupling diazotized aniline with aniline. Crystallization from pentane gave yellow crystals, m.p. 94-96° (142).

4-Aminoazobenzene was prepared by the rearrangement of 1,3-diphenyltriazene. The hydrochloride was purified by crystallisation from very dilute hydrochloric acid. The free amine was crystallised from aqueous alcohol and then heptane to give orange crystals, m.p. 124-125°. Lit. 127-128° (122), 124.5-125.4° (206).

4-Hydroxyazobenzene was formed by coupling diazotized aniline with phenol. Two crystallisations from heptane and one from benzene gave yellow crystals, m.p. 155-156°. Lit. 150° (224).

4-Methylaminoazobenzene was prepared by the rearrangement of 1,3-diphenyl-1-methyltriazene. Two crystallisations from hexane gave lustrous yellow-orange needles, m.p. 89-90°. Lit. 88.0-88.5° (206).

4-Dimethylaminoazobenzene was prepared by coupling diazotised aniline with dimethylaniline. Crystallisation from aqueous alcohol and then twice from pentane gave glossy orange crystals, m.p. 117-118°. Lit. 114° (212).

4-Methylethylaminoazobenzene was obtained by coupling diazotised aniline with methylethylaniline. Two crystallisations from heptane gave orange crystals, m.p. 72-73°. Lit. 69.5-71.5° (206).

4-Ethylaminoazobenzene was prepared by rearrangement of 1,3-

diphenyl-1-methyltriazene. Two crystallizations from heptane gave long red needles, m.p. 87-88°. Lit. 86.5-87.5° (206).

4-Diethylaminoazobenzene was synthesized by coupling diazotized aniline with diethylaniline. Crystallization twice from aqueous methanol, then pentane, followed by ethanol and finally pentane gave brown-yellow crystals, m.p. 97-98°. Lit. 96.5-98.0° (206).

4-Benzalaminoazobenzene. A mixture of 1 equivalent of 4-aminoazobenzene and 1 equivalent of benzaldehyde was refluxed for 5 minutes. The mixture solidified. Two crystallizations from ethanol gave orange plates (85-95%), m.p. 128-129°. Lit. 128° (29).

4-Benzylmethylaminoazobenzene was prepared by coupling diazotised aniline with benzylmethylaniline. Crystallisation from ethanol and then twice from hexane gave brown-orange needles, m.p. 99-100°. Lit. 99.5-100° (206).

2,4-Diaminoazobenzene was formed by coupling diazotised aniline with pure meta phenylenediamine. Crystallisation from heptane, water and then heptane again gave orange crystals, m.p. 118-119°. Lit. 117.5° (302).

3'-Methyl-4-methylaminoazobenzene. o-Toluidine was diazotised to give 3-o-tolyl-1-phenyl-1-methyltriazene. The triazene was then rearranged to give the desired compound. Crystallisation from aqueous methanol and then pentane gave golden-yellow cottony needles, m.p. 109-110°. Lit. 109-110° (206).

3'-Methyl-4-dimethylaminoazobenzene was obtained from Miss Mary Lois Jung, m.p. 120-121°. Lit. 119.5-120.5° (206).

2-Methyl-4-dimethylaminoazobenzene was obtained from Miss Mary Lois Jung, m.p. 68-69°. Lit. 66° (264).

2'-Methyl-4-dimethylaminoazobenzene was obtained from Miss Mary Lois Jung, m.p. 73-74°. Lit. 65-66° (199), 73.5-74.0° (207).

4'-Methyl-4-dimethylaminoazobenzene was prepared by Miss Mary Lois Jung, m.p. 170-171°. Lit. 169° (42).

4-Formylaminoazobenzene was made by refluxing 4-aminoazobenzene in excess formic acid for 2 hours. Yellow plates were formed from aqueous alcohol, m.p. 163-164°. Lit. 162.0-162.5° (206).

4-Methylformylaminoazobenzene was formed by refluxing 4-methylaminoazobenzene in excess formic acid for 2 hours. Crystallization from aqueous alcohol and then hexane gave yellow crystals, m.p. 111-112°. Lit. 110.5-111.5° (210).

2',4',6'-Tribromo-4-dimethylaminoazobenzene was prepared by coupling diazotized 2,4,6-tribromoaniline (284) with dimethylaniline. Crystallization from heptane gave large red crystals, m.p. 162-163°. Lit. 162.0-162.5° (210).

2',5'-Dimethyl-4-dimethylaminoazobenzene was prepared by coupling diazotized p-xylidine with dimethylaniline. Crystallization from methyl cellosolve and then heptane gave well-formed red crystals, m.p.

157-158°. Lit. 157.5-158.0° (206).

4-Acetylaminoazobenzene was prepared by the acetylation of 4-aminoazobenzene in hot benzene with acetic anhydride. Golden yellow cottony crystals were obtained from heptane, m.p. 151-152°. Lit. 141° (270).

4-Carbethoxyaminoazobenzene was formed by the action of ethyl chlorocarbonate on a cold pyridine solution of 4-aminoazobenzene. A practically quantitative yield of glistening yellow needles was obtained from heptane, m.p. 152-153° (192).

3,4'-Dimethyl-4-aminoazobenzene. The diazotization of p-toluidine gave 1,3-di-p-tolyltriazene. Reaction with o-toluidine gave the desired compound. Three crystallizations from aqueous alcohol and then two from hexane gave orange crystals, m.p. 130-131°. Lit. 128° (89).

4',5-Dimethyl-2-aminoazobenzene. 1,3-di-p-tolyltriazene was rearranged with p-toluidine and p-toluidine hydrochloride to give the desired amine. Three crystallizations from ethanol and one from pentane gave brown-red crystals, m.p. 118-119°. Lit. 119° (89).

2,2'-Dimethyl-4-dimethylaminoazobenzene was prepared by coupling diazotized o-toluidine with dimethyl-m-toluidine. Two crystallizations from aqueous methanol gave red-orange crystals, m.p. 82-83°.

Anal. Calcd. for $C_{16}H_{19}N_3$. N, 16.6. Found: N, 16.3.

2,4'-Dimethyl-4-aminoazobenzene. Diazotization of p-toluidine gave 1,3-di-p-tolyltriazene. Reaction of this derivative with m-toluidine hydrochloride gave the desired compound. Orange crystals were obtained from aqueous methanol, m.p. 127-128° . Lit. 128° (89,200).

2,4'-Dimethyl-4-dimethylaminoazobenzene was prepared by coupling diazotized p-toluidine with dimethyl-m-toluidine. Two crystallizations from methanol gave red crystals, m.p. 121-122° . Lit. 121° (264).

2,3'-Dimethyl-4-aminoazobenzene was prepared by the diazotization of m-toluidine. Three crystallizations from aqueous methanol and then one from hexane gave glistening orange plates, m.p. 81-82° . Lit. 81° (200).

2',3-Dimethyl-4-aminoazobenzene. The Eastman Kodak Co. white label product was crystallized from alcohol and then heptane to give large red-brown prisms, m.p. 101-102° . Lit 100° (89).

4-Tosylaminoazobenzene. This new compound was prepared by the addition of 2.1 g. of p-toluenesulfonyl chloride to a stirred ice-cold solution of 2.33 g. 4-aminoazobenzene hydrochloride in 40 ml. of pyridine. After 0.5 hour of stirring, the mixture was poured into excess ice-cold dilute hydrochloric acid. Crystallization from aqueous methyl cellosolve and then heptane gave glossy brown-yellow needles in 90-95% yield, m.p. 162-163° .

Anal. Calcd. for $C_{19}H_{17}N_3O_2S$: S, 9.12. Found: S, 9.08.

2-Benzalaminonaphthalene was formed by reaction between equimolar amounts of 2-naphthylamine and benzaldehyde in hot alcohol. Crystallization from hexane and then aqueous alcohol gave yellow needles, m.p. 102-103°. Lit. 102-103° (78).

1-Benzalaminonaphthalene was prepared by the same procedure as for the 2-isomer. Crystallization from alcohol gave light yellow platelets, m.p. 74°. Lit. 73.5° (249).

2-Benzoylaminonaphthalene was formed in practically quantitative yield by the reaction between benzoyl chloride and an equimolar amount of 2-naphthylamine in pyridine. Crystallization from heptane-benzene gave colorless crystals, m.p. 162-163°. Lit. 157° (22).

1-Benzoylaminonaphthalene was prepared by the same procedure as for the 2-isomer. Crystallization from hexane gave beautiful tufts of needles (85-95%), m.p. 159-160°. Lit. 161° (125).

2-Carbethoxyaminonaphthalene was formed by adding a slight excess of ethyl chlorocarbonate to a cold pyridine solution of 2-naphthylamine. Crystallization from hexane gave colorless needles (90-95%), m.p. 71-72°. Lit. 73° (86).

1-Carbethoxyaminonaphthalene was prepared by the same procedure as for the 2-isomer. Crystallization from hexane gave colorless needles in 90% yield, m.p. 79-80°. Lit. 79° (134).

2-Acetylaminonaphthalene was formed by refluxing acetic anhydride with a hot benzene solution of 2-naphthylamine for one hour.

Colorless plates (95%) were obtained from benzene-heptane, m.p. 134°. Lit. 132° (163).

1-Acetylaminonaphthalene was prepared by the same procedure as for the 2-isomer. Crystallization from benzene gave colorless needles, m.p. 158-159°. Lit. 160° (125).

2-(4'-Dimethylaminobenzeneazo)naphthalene was prepared by coupling diazotized 2-naphthylamine with dimethylaniline. Two crystallizations from heptane gave orange crystals, m.p. 174°. Lit. 174° (133).

1-(4'-Dimethylaminobenzeneazo)naphthalene was prepared by coupling diazotized 1-naphthylamine with dimethylaniline. Crystallization from heptane and then methyl cellosolve gave lustrous purple plates, m.p. 134-135°. Lit. 100° dec. (33).

2,2'-Azonaphthalene was prepared by diazotization of 2-naphthylamine. The crude product was extracted with boiling ethanol. The resultant residue was crystallized four times from xylene and then from methyl cellosolve to give red-gold plates, m.p. 207-208°. Lit. 202° (37).

1,1'-Azonaphthalene was prepared by diazotization of 1-naphthylamine. Three crystallizations from acetic acid gave orange crystals, m.p. 189-190°. Lit. 183-185° (37).

1-Anthramine was prepared by the reduction of 1-aminoanthraquinone with zinc dust in aqueous sodium hydroxide. Crystallization from aqueous methanol and then twice from hexane gave bright yellow plates, m.p. 122-123°. Lit. 121.5-122.5°(113), 127°(46).

1-Carbethoxyaminoanthracene. This new compound was prepared by adding 2.2 ml. of ethyl chlorocarbonate dropwise to a stirred solution of 3.86 g. of 1-anthramine in 50 ml. ice-cold pyridine. The solution was allowed to stand at room temperature for one hour and then poured into excess cold dilute hydrochloric acid. The air-dried precipitate was crystallized from heptane in yellow needles (96%), m.p. 140-141°.

Anal. Calcd. for $C_{17}H_{15}NO_2$: N, 5.28. Found: N, 5.23.

2-Carbethoxyaminoanthracene. This new compound was prepared in the same fashion as was the 1-isomer. Crystallization from aqueous alcohol and then heptane-toluene (6:1) gave light yellow plates (85-90%) m.p. 216°.

Anal. Calcd. for $C_{17}H_{15}NO_2$: N, 5.28. Found: N, 5.42.

2-Acetylaminoanthracene. To a hot solution of 1.93 g. 2-anthramine in 100 ml. xylene was added 1.1 ml. acetic anhydride. The solution was allowed to cool to room temperature. Yellow crystals (95-100%) were obtained, m.p. 240-243°. Two crystallizations from acetic acid gave yellow plates, m.p. 245°. The derivative can also be crystallized from methyl cellosolve or nitrobenzene. It has a blue fluorescence in solution. Lit. 240°(255).

2-Aminoanthracene was synthesized by the reduction of 2-aminoanthraquinone with zinc in 5% aqueous sodium hydroxide. Crystallization twice from xylene and then from alcohol gave yellow glistening plates, m.p. 244° dec. Lit. 238° (238). The compound can also be crystallized from a large volume of heptane. It has a green fluorescence in solution.

1-Tosylaminoanthracene. This new compound was obtained by the addition of 3.9 g. of p-toluenesulfonyl chloride to 3.84 g. of 1-aminoanthracene in 40 ml. of pyridine. The dark solution was refluxed for 0.5 hour and then poured into excess dilute ice-cold hydrochloric acid. Crystallization from methanol gave very light yellow needles (90-95%), m.p. 208°.

Anal. Calcd. for $C_{21}H_{17}NO_2S$: S, 9.22. Found: S, 9.18.

1-Methyltosylaminoanthracene. This new compound was formed by the addition of a solution of 0.14 g. potassium hydroxide in 10 ml. water and then 0.2 ml. methyl iodide to a warm solution of 0.83 g. 1-tosylaminoanthracene in 35 ml. alcohol. The mixture was refluxed for 1 hour and then poured into excess water. Crystallization from aqueous alcohol gave colorless crystals (90-95%), m.p. 173°.

Anal. Calcd. for $C_{22}H_{19}NO_2S$: S, 8.86. Found: S, 8.93.

2-Tosylaminoanthracene. This new compound was prepared by the same procedure used for the 1- isomer. Crystallization from methanol and then from aqueous acetic acid gave faint yellow microcrystals (80-85%), m.p. 186°.

Anal. Calcd. for $C_{21}H_{17}NO_2S$: S, 9.22. Found: S, 9.35.

2-Methyltosylaminoanthracene. This new compound was prepared by the same procedure used for the 1- isomer. Crystallization from methanol gave glistening faint yellow crystals (90-95%), m.p. 172-173°.

Anal. Calcd. for $C_{22}H_{19}NO_2S$: S, 8.86. Found: S, 9.01.

2-(4'-Nitrobenzenesulfonylamino)anthracene. This new compound was formed by refluxing for one hour 1.5 g. p-nitrobenzenesulfonyl chloride and 1.3 g. 2-anthramine in 20 ml. pyridine. The dark solution was then poured into excess ice-cold dilute hydrochloric acid. Crystallization from about 200 ml. alcohol gave dark yellow gleaming crystals (85-90%), m. p. 225-226°.

Anal. Calcd. for $C_{20}H_{14}N_2O_4S$: S, 8.47. Found: S, 8.52.

2-(Methyl-4'-nitrobenzenesulfonylamino)anthracene. This new compound was formed by the same procedure that was used for the preparation of 1-methyltosylaminoanthracene. Crystallization from alcohol gave light yellow crystals (90-95%), m.p. 203-204°. Acetic acid or benzene-heptane can also be used as crystallizing solvents.

Anal. Calcd. for $C_{21}H_{16}N_2O_4S$: S, 8.16. Found: S, 8.10.

1-Benzalaminoanthracene. This new compound was prepared by the addition of 2.2 ml. benzaldehyde to a boiling solution of 3.86 g. 1-anthramine in 70 ml. methanol. The solution was refluxed for 30 minutes, cooled and then filtered. The brown microcrystals were crystallized from heptane in flower-like clusters of dark yellow needles (85-90%), m.p. 174-176°.

Anal. Calcd. for $C_{21}H_{15}N$: C, 89.68; H, 5.34. Found: C, 89.50; H, 5.50.

2-Benzalaminoanthracene. This new compound was obtained by the same procedure that was used for the 1- isomer except that methyl cellosolve was used as the solvent. Crystallization from benzene-heptane (6:1) gave bright yellow crystals (80-90%), m.p. 213-214°. Methyl cellosolve, benzene, a large volume of heptane or a very large volume of alcohol can also be used as crystallizing solvents.

Anal. Calcd. for $C_{21}H_{15}N$: C, 89.68; H, 5.34. Found: C, 89.44; H, 5.66.

2-Aminobiphenyl. The Eastman Kodak Co. practical grade compound was crystallized three times from heptane to give colorless needles, m.p. 49°. Lit. 49-50° (265).

2-Acetylaminobiphenyl was obtained by the addition of slightly more than 1 equivalent of acetic anhydride to a hot benzene solution of 2-aminobiphenyl. Colorless needles were obtained from heptane, m.p. 121°. Lit 120° (263).

4-Aminobiphenyl. The Eastman Kodak Co. practical grade compound was crystallized several times from heptane in colorless crystals, m.p. 55°. Lit. 52-53° (23).

4-Acetylaminobiphenyl was prepared by the action of an equiv-slight amount of acetic anhydride on a suspension of 4-aminobiphenyl in benzene. Crystallization from xylene and then from aqueous methanol gave colorless needles, m.p. 170-171°. Lit. 172° (266).

4-Dimethylaminobiphenyl was obtained by the action of dimethyl-sulfate on molten 4-aminobiphenyl. Crystallization from aqueous methanol, pentane and then aqueous methanol gave colorless plates, m.p. 122-123°. Lit. 120-121° (209).

3-Nitro-4-acetylaminobiphenyl was obtained by the nitration of 4-acetylaminobiphenyl in acetic acid with fuming nitric acid. Crystallization from methanol and then from heptane gave long yellow needles, m.p. 132°. Lit. 132° (66,111).

3-Nitro-4-aminobiphenyl was obtained by refluxing concentrated hydrochloric acid with a methyl cellosolve solution of 3-nitro-4-acetylaminobiphenyl for several hours. Red needles were obtained from methanol, m.p. 167-168°. Lit. 167° (111).

4,4'-Diacetylaminobiphenyl was prepared by the action of 2.2 equivalents acetic anhydride on a hot solution of benzidine in xylene. Long colorless needles were formed from methyl cellosolve or nitrobenzene, m.p. 335-337° dec. Lit. 322° (47).

p-Terphenyl. The Eastman Kodak Co. technical grade product was crystallized from xylene, methyl cellosolve and then heptane in colorless plates, m.p. 212-213°. Lit. 212° (124).

4-Nitro-p-terphenyl was formed by the nitration of p-terphenyl in acetic acid. Crystallization from acetic acid and then from methyl cellosolve gave crystals melting at 205-210° . Lit. 210-214° (6).

4-Amino-p-terphenyl was prepared by the reduction of 4-nitro-p-terphenyl in alcohol with a hydrochloric acid solution of stannous chloride. Glistening silver plates from alcohol, m.p. 200° , showed a violet fluorescence in solution. Lit. 197-198° (116).

4,4''-Dinitro-p-terphenyl was formed in long yellow needles by the nitration of p-terphenyl in hot acetic acid with fuming nitric acid. Several crystallizations from nitrobenzene gave yellow needles, m.p. 275-276° . Lit. 272-273° (116). This compound forms dark complexes with 4,4''-diamino-p-terphenyl, 2,7-diaminofluorene and benzidine.

4,4''-Diamino-p-terphenyl. A melting point but no details of preparation are given in the literature (293). A hot solution of hydrated stannous chloride (30 g.) in 80 ml. concentrated hydrochloric acid was added to a hot solution of 7.0 g. of 4,4''-dinitro-p-terphenyl in 150 ml. methyl cellosolve. This mixture was refluxed for 3 hours. Colorless plates replaced the yellow needles. To a stirred suspension of the plates in 50 ml. methyl cellosolve was added concentrated ammonia. The alkaline mixture was heated to boiling. Hot water was added to a definite turbidity. After filtration, the solution was allowed to cool. Large colorless plates (5.2 g. 91%) were obtained. Crystallization from xylene, aqueous methyl cellosolve or aqueous acetone gave colorless plates, m.p. 248-249° , with a blue fluorescence in solution. Lit. 236° (293).

Anal. Calcd. for $C_{18}H_{16}N_2$: N, 10.77. Found: N, 10.54.

4-Carbethoxyamino-p-terphenyl. This new compound was prepared by the dropwise addition of 3.5 g. of ethyl chlorocarbonate to 7.2 g. of 4-amino-p-terphenyl in 50 ml. of stirred ice-cold pyridine. The cold mixture was stirred an additional 30 minutes and then poured into excess ice-cold dilute hydrochloric acid. Several crystallizations from aqueous methyl cellosolve gave a colorless wax-like product in 80-85% yield.

Anal. Calcd. for $C_{21}H_{19}NO_2$: N, 4.42. Found: N, 4.30.

3-Nitro-4-carbethoxyamino-p-terphenyl. This new compound was synthesized in approximately 75% yield by the dropwise addition of 1 ml. of fuming nitric acid (d. 1.5) to a stirred suspension of 1.1 g. of 4-carbethoxyamino-p-terphenyl in 10 ml. acetic acid a room temperature. The mixture was allowed to stand at room temperature for 3 hours and then filtered. Crystallization from a large volume of ethanol gave yellow needles, m.p. 171-172° . This compound can also be crystallized from heptane or acetic acid.

Anal. Calcd. for $C_{21}H_{18}N_2O_4$: N, 7.73. Found: N, 8.05.

Fluorene. The commercial product was crystallized from acetic acid and then heptane, m.p. 116-117° . Lit. 116-117° (12).

2-Nitrofluorene was obtained by the nitration of fluorene.

Crystallization from methyl cellosolve and then acetone gave faint yellow crystals, m.p. 157°. Lit. 157° (180). The compound can also be crystallized from heptane or acetic acid.

2-Aminofluorene was formed by the reduction of 2-nitrofluorene. Crystallization from hexane and then aqueous alcohol gave colorless needles, m.p. 129-130°. Lit. 127.5° (180). The amine was still colorless after one year in a dark drawer.

2-(4'-Dimethylaminobenzeneazo)fluorene was synthesized by coupling diazotized 2-aminofluorene with dimethylaniline. Crystallization twice from methyl cellosolve and then from acetone gave yellow crystals, m.p. 227°. Lit. 227° (177).

2-Acetylaminofluorene was prepared by the action of acetic anhydride on a boiling solution of 2-aminofluorene in benzene, heptane or carbon tetrachloride. Crystallization from alcohol, aqueous acetic acid or xylene gave colorless crystals, m.p. 194-195°. Lit. 190-191° (209).

2-Carbethoxyaminofluorene was formed by the dropwise addition of ethyl chlorocarbonate to an equivalent amount of 2-aminofluorene in ice-cold pyridine. Crystallization from heptane gave colorless plates (85-90%), m.p. 121-122°. Lit. 121-122° (63).

2-Benzalaminofluorene was obtained by adding benzaldehyde to an equivalent amount of 2-aminofluorene in hot alcohol. Crystallization from heptane, ethanol or acetone gave yellow plates (90-95%), m.p. 159-160°. Lit. 152° (238).

2-(4'-Nitrobenzylamino)fluorene was prepared by the action of p-nitrobenzyl chloride on an equivalent amount of 2-aminofluorene in boiling pyridine. Crystallization from aqueous methyl cellosolve gave large red crystals, m.p. 149-150°. Lit. 141.8-143.2° (292).

2-Benzoylaminofluorene was prepared by the action of benzoyl chloride on an equivalent amount of 2-aminofluorene in pyridine. Two crystallizations from acetic acid gave colorless needles (80-90%), m.p. 217-218°. Lit. 215° (13).

2,7-Dinitrofluorene was prepared by the nitration of fluorene. Crystallization from methyl cellosolve gave long slender yellow needles, m.p. 295-300° dec. Lit. 334° (68). Acetic acid and nitrobenzene can also be used as crystallizing solvents.

2,5-Dinitrofluorene was isolated as a byproduct in the preparation of the 2,7- isomer. Two crystallizations from acetic acid gave yellow needles, m.p. 207°. Lit. 207° (68).

2,7-Diaminofluorene was obtained by the reduction of 2,7-dinitrofluorene. Crystallization from aqueous alcohol gave colorless needles, m.p. 164-165°. Lit. 163-164° (268). This compound can also be crystallized from heptane-acetone in colorless plates.

2,5-Diaminofluorene was synthesized by the reduction of 2,5-dinitrofluorene with sodium hydrosulfite. Crystallization from benzene gave a melting point of [illegible]° . Lit. 175° (213).

2,7-Diacetylaminofluorene was formed by the acetylation of the diamine. Crystallization from aqueous acetic acid followed by extraction of impurities with boiling acetone gave colorless crystals m.p. 289-290° . Lit. 291° (213).

2,5-Diacetylaminofluorene was formed by acetylating 2,5-diaminofluorene in hot benzene with 2 equivalents of acetic anhydride. Two crystallizations from aqueous acetic acid gave colorless crystals, m.p. 294-295° . Lit. 289° (213).

2-Hydroxyfluorene was prepared by the hydrolysis of diazotized 2-aminofluorene. Crystallization from heptane gave glossy colorless crystals melting at 171° . Lit. 169-171° (248).

2-Acetoxyfluorene was prepared by the acetylation of 2-hydroxyfluorene with excess hot acetic anhydride in the presence of anhydrous sodium acetate. Crystallization from heptane gave colorless needles, m.p. 129° . Lit. 128° . (77).

2-Bromo-7-acetylaminofluorene was obtained by the bromination of 2-acetylaminofluorene. Crystallization from xylene and then acetic acid gave crystals melting at 225-226° . Lit. 228° (24).

1,3,7-Tribromo-2-aminofluorene was formed by the bromination of 2-aminofluorene. Crystallization from aqueous acetic acid, heptane and then alcohol gave colorless cottony crystals, m.p. 200° . Lit.

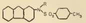
m.p. 205° (24).

2-Tosylaminofluorene. To a solution of 5.4 g. of 2-aminofluorene in 40 ml. boiling acetic acid was added 9.5 g. of p-toluenesulfonyl chloride. To this boiling milky mixture was added 4.1 g. of sodium acetate in small portions over 15 minutes. The mixture was then refluxed for 15 minutes. Finally water was added slowly to the boiling solution until crystals started to come out. The mixture was allowed to cool and was then filtered. The large crystals were recrystallized from aqueous acetic acid. Yield of colorless crystals, melting at 162° , was 8.0 to 8.5 g. (80-85%). Lit. 161° (24). The compound can also be crystallized from methyl alcohol.

General Preparation of the 2-Tosylaminofluorenes. To 0.01 mole of 2-tosylaminofluorene dissolved in 100 ml. of warm neutral ethyl alcohol was added 10 ml. of 1 N aqueous sodium hydroxide followed by 0.01 mole of the halide. The mixture was refluxed for one hour. Then 200 ml. of water was added. After cooling, the mixture was filtered. Sometimes an oil formed; in that case, the mixture was allowed to stand overnight in the cold room and then filtered. Recrystallizing solvent in all cases was methyl alcohol. Yields, melting points and analyses are reported in Table VI. The alkyltosylaminofluorenes form yellow colors in sulfuric acid which quickly change to violet.

TABLE VI

NEW 2-TOSYLAMINOFLUORENE DERIVATIVES



RX	Yield	m.p.	Analyses % N Calcd.	 Found	 % S Calcd.	 Found
Methyl iodide*	95	137-138°				
Ethyl iodide	95	135°	3.86	3.92	8.81	8.80
n-Propyl bromide	92	125-126°	3.71	3.67	8.49	8.52
n-Butyl bromide	65	132-133°	3.58	3.65	8.18	8.10
Benzyl chloride	93	200°	3.29	3.31	7.53	7.50
Allyl bromide	78	131-132°	3.73	3.57	8.53	8.36
Methylallyl bromide	74	175-176°	3.60	3.81	8.23	8.32
Ethyl chlorocarbonate	50	193°	3.44	3.37	7.86	8.05
Acetyl chloride	75	164-165°	3.71	3.64	8.49	8.35

* Ray, F. E. and Little, J., In Press.

__2-(2-Hydroxybenzalamino)fluorene__. This new compound was prepared by the addition of salicaldehyde to an equivalent amount of 2-aminofluorene in hot alcohol. The precipitated yellow crystals were crystallized from alcohol and then heptane in 70-75% yield, m.p. 156-157°. The procedure was the same as for the 4' isomer.

__Anal__. Calcd. for $C_{20}H_{15}NO$: N, 4.91. Found: N, 4.80.

__2-(4'-Hydroxybenzalamino)fluorene__. This new compound was synthesized by adding 2.5 g. of p-hydroxybenzaldehyde to a boiling solution of 3.6 g. 2-aminofluorene in 40 ml. alcohol. The mixture was refluxed for 15 minutes, allowed to cool and filtered. Two crystallizations from xylene gave yellow plates (65-70%), m.p. 242-243°.

__Anal__. Calcd. for $C_{20}H_{15}NO$: N, 4.91. Found: N, 4.69.

__2-(2',4'-Dihydroxybenzalamino)fluorene__. This new compound was prepared by the same procedure as was used for the 2-(4'-hydroxybenzalamino)fluorene. Crystallization from xylene, alcohol and then xylene gave yellow crystals (60-70%), m.p. 202-203°.

__Anal__. Calcd. for $C_{20}H_{15}NO_2$: N, 4.65. Found: N, 4.41.

__2-Cinnamalaminofluorene__. This new compound was formed by adding a hot solution of 6.6 g. of cinnamaldehyde in 60 ml. alcohol to a hot solution of 9.0 g. 2-aminofluorene in 50 ml. alcohol. The solution solidified. This mixture was refluxed for 15 minutes and then allowed to cool. Crystallization from methyl cellosolve and then benzene gave yellow needles (70-80%), m.p. 181-182°. The compound can also be crystallized from a large volume of alcohol or from an even larger volume of heptane. It forms a yellow solution in concentrated sulfuric acid.

Anal. Calcd. for $C_{22}H_{17}N$: N, 4.75. Found: N, 4.55.

2-Nitro-9-benzalfluorene was prepared by the reaction between 2-nitrofluorene and an equivalent amount of benzaldehyde in boiling methyl cellosolve in the presence of a few drops of alcoholic sodium ethoxide. Crystallization from aqueous methyl cellosolve and then from xylene-heptane gave yellow crystals, m.p. 148-173°. This is evidently a mixture of the cis and trans isomers. Lit. 149°. (190).

2-Nitro-9-(4'-dimethylaminobenzal)fluorene was obtained by refluxing 2-nitrofluorene and an equivalent amount of p-dimethylaminobenzaldehyde in methyl cellosolve containing a small amount of sodium ethoxide. Two crystallizations from xylene gave deep red crystals, m.p. 204-206°. Lit. 204° (190).

2-Nitrofluorenone was made by the oxidation of 2-nitrofluorene. Crystallization from acetic acid gave yellow needles, m.p. 219°. Lit. 218-219° (98). The compound forms a red solution in sulfuric acid.

2-Aminofluorenone was synthesized by the reduction of 2-nitrofluorenone with sodium sulfide in alcoholic sodium hydroxide solution. Crystallization from aqueous alcohol and then heptane gave black-violet needles, m.p. 155-156°. Lit. 160° (98). Water can also be used as a crystallizing solvent.

2-Acetylaminofluorenone was formed by the acetylation of 2-aminofluorenone in hot benzene with an equivalent amount of acetic anhydride. Crystallization from alcohol gave bright orange needles, m.p. 230° . Lit. 229-230° (168).

2,7-Diacetyl-9,9-di-β-propionitrilefluorene. This new compound was synthesized by adding 1.1 g. of acrylonitrile and then several drops of alcoholic sodium ethoxide to a solution of 2.8 g. of 2,7-diacetylfluorene in 20 ml. of boiling methyl cellosolve. A dark blue color was formed and then a crystalline precipitate came down. The mixture was refluxed for 0.5 hour. Excess 5% acetic acid was added to the ice-cold mixture. The red-brown precipitate was collected on a Buchner funnel and washed with water. Crystallization from acetic acid gave colorless crystals (80-85%), m.p. 272-273° . Xylene, butyl alcohol or methyl cellosolve can also be used as crystallizing solvents. This compound and 2,7-diacetyl fluorene have a purple fluorescence in sulfuric acid solution.

Anal. Calcd. for $C_{23}H_{20}N_2O_2$: N, 7.87. Found: N, 7.57.

2-(4'-Dimethylaminobenzalamino)fluorene. This new compound was prepared by refluxing 1.5 g. of p-dimethylaminobenzaldehyde with 1.8 g. of 2-aminofluorene in 20 ml. alcohol. Crystallization from butyl alcohol and then xylene gave yellow microcrystals in 70-73% yield, m.p. 211-212° .

Anal. Calcd. for $C_{22}H_{20}N_2$: N, 8.97. Found: N, 8.80.

9,9'-Bifluorene was made by the controlled dehydrogenation of fluorene with sulfur. Crystallization from benzene and then heptane gave colorless needles, m.p. 247°. Lit. 236° (134), 247° (234).

2-(4'-Nitrobenzenesulfonylamino)fluorene. This new compound was obtained by the action of 6.7 g. of p-nitrobenzenesulfonyl chloride on 5.4 g. of 2-aminofluorene in 30 ml. pyridine. The dark violet solution was refluxed for 0.5 hour and then poured into ice-cold dilute hydrochloric acid. Crystallization from aqueous acetic acid and then benzene gave light yellow crystals (80-85%), m.p. 205-206° to a red liquid. The compound forms an orange solution in alcoholic sodium hydroxide.

Anal. Calcd. for $C_{19}H_{14}N_2O_4S$: N, 7.65. Found: N, 7.75.

2-(Methyl-4'-nitrobenzenesulfonylamino)fluorene. This new compound was synthesized in 96% yield by the procedure used for the preparation of the alkyltosylaminofluorenes. Crystallization from alcohol gave colorless cottony crystals, m.p. 185°.

Anal. Calcd. for $C_{20}H_{16}N_2O_4S$: S, 8.42. Found: S, 8.40.

Dibenzofuran. The Eastman Kodak Co. technical grade product was crystallized twice from methanol and then twice from hexane, m.p. 83-84°. Lit. 87° (90).

3-Nitrodibenzofuran was prepared by the nitration of dibenzofuran. Crystallization from alcohol and then heptane gave colorless crystals, m.p. 183°. Lit. 181-182° (40).

3-Aminodibenzofuran was prepared by the reduction of 3-nitrodibenzofuran. Crystallization from dilute alcohol and then heptane gave colorless crystals, m.p. 99°. Lit. 94° (90,129).

3-Acetylaminodibenzofuran was formed by the acetylation of the amine in hot benzene with acetic anhydride. Crystallization from alcohol and then xylene gave colorless crystals, m.p. 179-180°. Lit. 179-180° (209).

3,3'-Azodibenzofuran was prepared by the reduction of 3-nitrodibenzofuran in boiling amyl alcohol with sodium amylate. Yellow cottony needles from methyl cellosolve or nitrobenzene, m.p. 282°, were soluble in sulfuric acid to a violet-red color. Lit. 282° (41).

2-Nitro-3-aminodibenzofuran was formed by hydrolysis of the acetyl derivative in a hot methyl cellosolve-hydrochloric acid solution. Crystallization from methyl cellosolve and then xylene gave orange needles, m.p. 222-223°. Lit. 222° (41).

2-Nitro-3-acetylaminodibenzofuran was formed by the nitration of 3-acetylaminodibenzofuran. Yellow needles were obtained from aqueous acetic acid or methyl cellosolve, m.p. 194-195°. Lit. 196°; 205° (1, 126).

2,3-Diaminodibenzofuran was prepared by reduction of 3-amino-2-nitrodibenzofuran in hot alcohol with a hydrochloric acid solution of stannous chloride. Colorless plates were formed from heptane, m.p. 166°. Lit. 164-166° (126).

Benzofuro(2,3-f)piaselenole. This new compound was prepared by adding 0.28 g. selenium dioxide to a hot solution of 0.6 g. of 2,3-diaminodibenzofuran in 30 ml. methanol. The resulting solution turned red and then after a short violent reaction, the mixture solidified to a yellow mush. Yellow needles (0.56 g. 66%) were obtained from methanol, m.p. 213°. The compound dissolves in concentrated sulfuric acid to a dark brown-yellow color.

Anal. Calcd. for $C_{12}H_6N_2OSe$: N, 10.23. Found: N, 10.31.

Dibenzothiophene was obtained by reaction between biphenyl, sulfur and aluminum chloride at high temperature. Crystallization from alcohol, acetic acid and then pentane gave colorless needles, m.p. 99°. Lit. 99° (127).

2-Nitrodibenzothiophene was formed by the nitration of dibenzothiophene. Two crystallizations from benzene gave yellow needles, m.p. 188-189°. Lit. 186-187° (130). The compound can also be crystallized from alcohol or heptane.

2-Aminodibenzothiophene was formed by the reduction of 2-nitrodibenzothiophene in hot acetic acid with a concentrated hydrochloric acid solution of stannous chloride. Crystallization from aqueous alcohol and then heptane gave colorless needles, m.p. 132-133°. Lit. 125-126°(130).

2-Carbethoxyaminodibenzothiophene. This new compound was prepared by a procedure similar to that given for the analogous dibenzoselenophene derivative. Crystallization from methanol and then heptane gave colorless needles (70%), m.p. 139-140°.

Anal. Calcd. for $C_{15}H_{13}NO_2S$: N, 5.16. Found: N, 5.13.

Dibenzothiophene-5-dioxide was formed in practically quantitative yield by the oxidation of dibenzothiophene in hot acetic acid with 30% hydrogen peroxide. Crystallization from alcohol gave colorless needles, m.p. 233-234°. Lit. 233-234° (128). It can also be crystallized from benzene, acetone, acetic acid or concentrated nitric acid.

Dibenzothiophene-5-oxide was formed by the oxidation of dibenzothiophene with chlorine. Crystallization from benzene and then alcohol gave large colorless needles, m.p. 188-189°. Lit. 185-187° (55).

3-Nitrodibenzothiophene-5-oxide was formed by the nitration of dibenzothiophene-5-oxide. Two crystallizations from alcohol gave the pure compound, m.p. 210-211°. Lit. 209.5-210.5° (55).

3-Aminodibenzothiophene was prepared by the reduction of 3-nitrodibenzothiophene-5-oxide. Crystallization from dilute alcohol and then heptane gave colorless crystals, m.p. 124-125°. Lit. 121-122.5° (55).

3-Acetylaminodibenzothiophene was prepared by the acetylation of the amine in benzene with acetic anhydride. Crystallization from benzene gave colorless crystals, m.p. 199-200°. Lit. 196-197° (55).

3-Carbethoxyaminodibenzothiophene. This new compound was formed by adding 13 g. of ethyl chlorocarbonate dropwise to a stirred ice-cold solution of 10 g. 3-aminodibenzothiophene in 100 ml. pyridine. The mixture was stirred an additional hour and then poured into dilute hydrochloric acid. A yellow oil precipitated which solidified on standing. Crystallization from heptane gave colorless crystals (80-85%), m.p. 124-125°.

Anal. Calcd. for $C_{15}H_{13}NO_2S$: N, 5.16. Found: N, 5.02.

2-Benzalaminodibenzothiophene. This new compound was prepared by refluxing 0.26 g. of 2-aminodibenzothiophene in 4 ml. alcohol with 0.15 ml. of benzaldehyde for 15 minutes. Solidification took place when the cooled supersaturated solution was scratched or seeded. Crystallization from aqueous ethanol gave colorless plates (80-85%), m.p. 121-122°.

Anal. Calcd. for $C_{19}H_{13}NS$: S, 11.2. Found: S, 11.0.

3-Benzalaminodibenzothiophene. This new compound was prepared by refluxing 0.3 g. of 3-aminodibenzothiophene in $\overset{5}{6}$ ml. of alcohol with 0.15 ml. benzaldehyde for 10 minutes. Yellow crystals (96%) were obtained, m.p. 159-160°. Crystallization from approximately 120 ml. hexane gave yellow needles, m.p. 160°.

Anal. Calcd. for $C_{19}H_{13}NS$: S, 11.2. Found: S, 11.1.

3-(4'-Dimethylaminobenzalamino)dibenzothiophene. This new compound was synthesized by refluxing for 1 hour 0.4 g. of 3-aminodibenzothiophene in 5 ml. alcohol with 0.28 g. of p-dimethylaminobenzaldehyde. Excess water was added. Two crystallizations from methyl cellosolve gave yellow crystals (80-85%), m.p. 195-196°.

Anal. Calcd. for $C_{21}H_{18}N_2S$: S, 9.70. Found: S, 9.5.

Di-o-biphenyldiselenide was prepared from 2-aminobiphenyl via 2-selenocyanobiphenyl. Several crystallizations from acetone gave large canary yellow crystals, m.p. 101°. Lit. 100-101° (198).

Dibenzoselenophene. The bromination of di-o-biphenyldiselenide gave o-biphenylselenenyl bromide which was ring-closed to dibenzoselenophene by heat. Vacuum distillation followed by crystallization from hexane gave colorless crystals, m.p. 76-78°. Lit. 73° (198). The compound was soluble in sulfuric acid to a blue color. It formed an orange-yellow picrate, m.p. 128-129°.

Dibenzoselenophene-5-oxide was obtained by the oxidation of dibenzoselenophene with peracetic acid. Crystallization from water and drying at 120° overnight gave colorless needles, m.p. 233-234°. Lit. m.p. 229-230° (198).

2-Nitrodibenzoselenophene. This new compound was formed by the dropwise addition of 1.5 ml. of fuming nitric acid (d. 1.5) to a stirred solution of 3.4 g. of dibenzoselenophene in 20 ml. acetic acid kept at 60-70°. Within several minutes the clear dark yellow solution had solidified to a yellow mush. The mixture was cooled to 20° and then filtered. Crystallization from alcohol yielded 1.6 g. (40%) of lustrous yellow needles, m.p. 180°. The compound dissolved in concentrated sulfuric acid with a light yellow color which turned red on heating.

Anal. Calcd. for $C_{12}H_7NO_2Se$: C, 52.20; H, 2.56; N, 5.07. Found: C, 52.26; H, 2.56; N, 5.08.

2-Aminodibenzoselenophene. This new compound was synthesized by the addition of a solution of 16.5 g. hydrated stannous chloride in 90 ml. concentrated hydrochloric acid to a solution of 3.6 g. of 2-nitrodibenzoselenophene in 90 ml. acetic acid. The mixture was refluxed several hours. The colorless suspension was cooled and then filtered. To a stirred suspension of the crystals in cold water, ammonium hydroxide was added until the mixture was definitely alkaline. Crystallization from hexane gave 2.5 g. (78%) of colorless crystals, m.p. 99°. The compound was soluble in sulfuric acid to a blue color.

Anal. Calcd. for $C_{12}H_9NSe$: N, 5.69. Found: N, 5.77.

2-Acetylaminodibenzoselenophene. This new compound was made by adding 0.2 ml. acetic anhydride to a hot solution of 0.5 g. of 2-aminodibenzoselenophene in 10 ml. benzene. In a few minutes the solution solidifies. After several hours standing, the mixture was filtered. Glistening colorless needles, melting at 181-182°, were obtained. Addition of hexane to the mother liquid gave more product. A total yield of 0.54 g. (92%) was obtained. Crystallization from heptane-benzene gave colorless needles, m.p. 182°. The compound was soluble in sulfuric acid to a blue color.

Anal. Calcd. for $C_{14}H_{11}NOSe$: N, 4.86; Se, 27.4. Found: N, 4.82; Se, 27.4.

2-Carbethoxyaminodibenzoselenophene. This new compound was prepared by adding 1.2 ml. of ethyl chlorocarbonate to an ice-cold solution of 2.0 g. of 2-aminodibenzoselenophene in 40 ml. pyridine. After one hour at 0-10°, excess cold dilute hydrochloric acid was added. This mixture was allowed to stand for several hours at room temperature so as to allow the oil to solidify. Crystallization from hexane gave colorless matted needles, m.p. 141°. The compound was soluble in concentrated sulfuric acid to a blue color.

Anal. Calcd. for $C_{15}H_{13}NO_2Se$: N, 4.40; Se, 24.8. Found: N, 4.49; Se, 24.7.

2-Benzalaminodibenzoselenophene. This new compound was prepared by adding 0.2 ml. benzaldehyde to a solution of 0.4 g. of 2-aminodibenzoselenophene in 10 ml. alcohol. The mixture was refluxed for one hour. Excess water was added. Crystallization from aqueous methyl cellosolve gave yellowish plates (85-90%), m.p. 133°. The compound was soluble in sulfuric acid to a yellow, changing to green, color.

Anal. Calcd. for $C_{19}H_{13}NSe$: Se, 23.7. Found: Se, 23.5.

2,5-Dimethyl-3-hexyne-2,5-diol was prepared by a modification of the procedure of Kazarian (164).

To a stirred solution of 112 g. of potassium hydroxide in 340 ml. of ether, 200 g. of calcium carbide was added. To this cooled (0-10°) and stirred mixture, 116 g. of acetone was added dropwise (1 hour). The temperature was allowed to rise to room temperature while the mixture was stirred seven more hours. The yellow mixture was allowed to stand overnight at room temperature. At this point it had solidified and was red-brown in color. Two hundred g. of ice was added and the solid was broken up. Then the ice-cold mixture was stirred for one hour and finally poured into a 4 liter stainless steel beaker. While stirring the yellow mixture by hand, dry ice (660 g., 15 moles) was added in small pieces. The mixture soon solidified. Ether (100-200 ml.) was added. The contents were then thoroughly stirred. The ether was decanted into a liter Erlenmeyer flask containing anhydrous calcium chloride. This process was repeated until 2 liters of ether had been thus used. The ether solutions were left overnight to dry and then distilled off. A brown oil was obtained. The oil was fractionally distilled under a vacuum. The fraction boiling at 65-135° under 1-2 mm. pressure was collected. Three hundred ml. of cold pentane was added to this half crystallized mixture. The colorless crystals were collected and rinsed with cold pentane. The yield of product, melting at 95-96°, was 47 g. The compound can be crystallized from heptane, benzene, toluene, xylene, carbon tetrachloride or chloroform.

The dibenzoate, a new compound, was prepared by the dropwise addition of 2.9 g. (0.02 mole) of benzoyl chloride to a stirred solution of 1.42 g. (0.01 mole) of the above mentioned diol in 5 ml. of pyridine. The dibenzoate melted at 112-113° after crystallization from alcohol.

Anal. Calcd. for $C_{11}H_{22}O_4$: C, 75.4; H, 6.29. Found: C, 75.3; H, 6.30.

2,5-Dimethyl-1,5-hexadiene-3-yne was prepared by the method of Mitchell and Marvel (211).

2,5-Diphenyl-3-hexyne-2,5-diol. The same procedure was followed as for the preparation of 2,5-dimethyl-3-hexyne-2,5-diol, except for the following modifications. Instead of acetone, 240 g. (2 moles) of acetophenone was used. The reaction took place readily at room temperature but would not take place to any great extent at 0-10°. The ether extract of the reaction mixture contained a large amount of a yellow precipitate of the higher melting stereoisomer. To the residual dark oil obtained after the ether was distilled off, 200-300 ml. of pentane was added. The mixture was allowed to stand overnight. It was then filtered and washed with pentane. The yield of yellow product was 130-150 g. (49-56%). Crystallization from 2250 ml. of xylene (Dorco) gave 120-140 g. (45-53%) of colorless crystals melting at 124-155° after washing with pentane. This is a mixture of the two stereoisomers. They can be separated by fractional crystallization from ether into an 'ether-insoluble' product melting at 164.0-165.5° and an ether-soluble isomer melting at 124-127°(101). Both of the isomers formed a violet solution in concentrated sulfuric acid.

2,5-Diphenyl-1,5-hexadiene-3-yne. The procedure was patterned after that of 2,5-dimethyl-1,5-hexadiene-3-yne. This new dienyne was prepared by refluxing 2,5-diphenyl-3-hexyne-2,5-diol (m.p. 124-155°) (47.9 g., 0.18 mole) and 500 ml. of 60% phosphoric acid for 90 minutes. Then the mixture was cooled. The phosphoric acid was poured off and the red-brown, britale, resinous material was washed by decantation until the washings were neutral. This material was dried and dissolved in about 150 ml. of boiling benzene (Darco) and filtered. On cooling, about 10 g. (24%) of yellow needles were obtained. Three crystallizations from xylene gave light yellow needles, m.p. 240°. The compound was soluble in chloroform, carbon tetrachloride and acetophenone, and insoluble in alcohol and acetone.

Anal. Calcd. for $C_{18}H_{14}$: C, 93.9; H, 6.1. Found: C, 93.64; H, 6.1.

1,1'-Dicyclohexanolacetylene. When the same procedure was used for the preparation of this compound as for 2,5-dimethyl-3-hexyne-2,5-diol, a yield of 25-30% of product was obtained. The procedure of Schwartzman (271) gave a higher yield. The compound was soluble in acetic acid, benzene, ether, acetone, alcohol and boiling heptane. One g. of the substance was soluble in approximately 125 ml. of boiling water. It formed a red-yellow solution in concentrated sulfuric acid. From aqueous acetic acid, water or heptane long white needles were obtained, m.p. 109-110°. Lit. 109-110° (271). A byproduct, $C_{16}H_{24}O_2$, was also obtained with the following method. In the first procedure after the ether extraction the mushy residue was filtered. The residue was suspended in 200-400 ml. of acetone and stirred vigorously. The mixture was filtered. The filtrate was added to 2 liters of water and allowed to stand overnight. The precipitate was crystallized from acetic acid. From 2 moles of cyclohexanone approximately 5-12 g. of white needles melting at 187-188° were obtained. Using Schwartzman's procedure in preparing the acetylene glycol it was found that when the benzene layer was evaporated carefully, the same compound was obtained melting at 185-186°. The compound was not very soluble in cold ether or hot water but was soluble in benzene or acetic acid. It formed a yellow solution in concentrated sulfuric acid. On reaction with benzoyl chloride and pyridine it was recovered unchanged; on reaction with 2,4-dinitrophenylhydrazine only starting products were isolated. Refluxing one gram of this compound for one hour with 50 ml. of 50% sulfuric acid gave a violet oil, from which mixture a small amount of colorless oil could be separated by steam distillation. This latter product has not been further investigated as yet.

Anal. Calcd. for $C_{16}H_{24}O_2$: C, 77.42; H, 9.68; mol. wt. 248. Found: C, 77.9; H, 9.31; mol. wt. (Rast, using camphor), 253.

1,1'-Dicyclohexenylacetylene was prepared by dehydration with 40% sulfuric acid (271) or potassium bisulfate (222).

Maleimide. Eighty ml. (1.13 mole) of pyrrole in 200 ml. of ether was added dropwise over three hours to a stirred cool solution of 520 g. sodium dichromate in 2400 ml. of water and 400 g. of concentrated sulfuric acid. The mixture was stirred vigorously with 200-300 ml. of ether at a time and then the ether layer, which contained the maleimide, was drawn off. The operation was repeated until a small amount of the ether extract gave no residue on evaporation in a watch glass. The dried ether extract was distilled until only a 100 ml. of ether solution remained. This residual solution was allowed to evaporate to dryness in the air. The blackish residue (15-20 g.) was recrystallized twice from a mixture of ethyl acetate and heptane (Darco) and finally crystallized from heptane. Approximately 5-10 g. (5-9%) of colorless needles were obtained melting at 93.5-94.5°. Lit. 93° (235). Maleimide is soluble in ether, acetone, water, ethyl acetate, alcohol and chloroform. It is not very soluble in benzene or heptane and is moderately soluble in hot carbon tetrachloride and soluble to a slight extent in boiling heptane.

9,10-Dihydroanthracene-9,10-endo-α,β-succinimide, a new compound, crystallized in a practically quantitative yield as colorless crystals from a solution of 1.0 g. anthracene and 0.6 g. maleimide in 6 ml. of xylene which had been gently refluxed for two to three minutes. After plunging in a bath at 320° it melted at 327-328°. It can be crystallized from aqueous methyl cellosolve or xylene.

Anal. Calcd. for $C_{18}H_{13}NO_2$: N, 5.09. Found: N, 5.29.

3,6-Dimethyl-3,6-endoxo-1,2,3,6-tetrahydrophthalimide, a new compound, was obtained in a quantitative yield as colorless plates, m.p. 116-117°, when 2.4 g. (0.025 moles) of freshly distilled 2,5-dimethylfuran was added to an ice- cold solution of 1.94 g. (0.02 mole) of maleimide in 10 ml of ether.

Anal. Calcd. for $C_{10}H_{11}NO_3$: N, 7.25. Found: N, 7.34.

Δ6a7a,1a12a-Tetradecahydrochrysene-5,6,11,12-tetracarbondiimide. This new compound was synthesized by the reaction of 1.0 g. (0.01 mole) of maleimide with 1.0 g. (0.0053 mole) 1,1'-dicyclohexenylacetylene in 15 ml. xylene. A few crystals of hydroquinone were added and the mixture was refluxed for seven hours. On cooling and filtering 1.0 g.

of crude product was obtained. Recrystallization from aqueous methyl cellosolve gave 0.6 g. colorless microcrystals melting at 357-358° with gas evolution (placed in bath at 350°).

Anal. Calcd. for $C_{22}H_{24}N_2O_4$: N, 7.36. Found: N, 7.30.

B. Measurement of the Absorption Spectra

1. Preparation of solutions. The pure compound (10^{-4} mole) was weighed with an accuracy of ± 0.1 mg. It was transferred quantitatively to a 100 ml. volumetric flask and diluted to the mark with filtered commercial 95% ethyl alcohol. A 5 ml. aliquot of the solution was transferred to another 100 ml. volumetric flask and diluted to the mark with alcohol. A spectral absorption curve was determined on the 5×10^{-5} M solution the same day. If the compound had a molecular weight less than 200, 2.5×10^{-4} mole of it was weighed out and diluted to 250 ml. For slightly soluble compounds, a larger volumetric flask was used. In order to avoid errors caused by changes in the temperature of the solution, the adjustment of the volume was delayed until the solution had attained the room temperature of 26 ± 1°. When the optical density at the wave length maximum was greater than 1.4 or less than 0.2, a new solution was substituted which would give readings in the 0.4 to 1.0 range. For all dilutions, pipettes and volumetric flasks had a minimum volume of 5 ml. and 50 ml., respectively.

2. Instrument operation. All absorption spectra were measured by means of a Beckman quartz photoelectric spectrophotometer Model DU, at a temperature of 26 ± 1°. The blank consisted of filtered commercial 95% ethyl alcohol. This alcohol (from the same bottle) was used in making up all the solutions. Silica cells were used for the whole wave length range. The instrument was balanced at an initial wave length of 2150 A. and an initial slit width of 2.0 mm. For

measurement at other wave lengths, the 'sensitivity' (set at about 1 to 3 turns from the counter clockwise limit) was not changed, while the slit width was varied for each wave length measurement to balance the instrument. The frequency at which readings of optical density were taken depended upon the complexity of the spectral curve; for sharp maxima and minima, readings were taken at intervals of 10 A. for about 40 A. on either side of the maximum or minimum, whereas in other regions of the curve, intervals of 20 to 50 A. were used. Proper functioning of the instrument was checked with a standard potassium acid phthalate solution from time to time by the method of Ewing and Parsons (105).

3. Plotting of spectral curves. In this paper, the various spectrophotometric terms used are defined as follows:

The optical density, E, is $\log (I_0/I)$, where I_0 equals the intensity of the incident light and I the intensity of the transmitted light.

The molecular extinction coefficient, ϵ, is defined as E/lc, where E is the optical density, l is the length of solution in centimeters traversed by the light, and c is the concentration in moles per liter.

In all the spectral curves the log of the molar extinction coefficient was plotted against the wave length in millimicrons. The reason for this choice was that curves for all types of compounds could be plotted over the whole spectral range of 2150 to 6000 A. on one type of graph. The coordinate scale was selected so that in the abscissa 200 A. equalled 1 cm. and in the ordinate 0.2 of the intensity unit equalled 1 cm. On this basis the spectral curves could be compared by superimposition. The vast majority of the spectral curves were determined on compounds that were prepared by the author. References are given for the curves taken from the literature.

FIG. 2

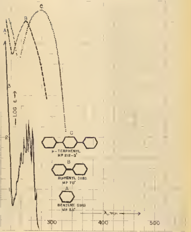

A
B
C
3
— LOG ε →
2
1
C
p-TERPHENYL
MP 212–3°
B
BIPHENYL (118)
MP 70°
A
BENZENE (196)
MP 5.5°
λ, mμ
300
400
500

FIG 3

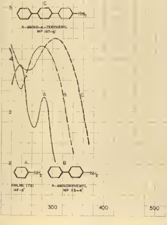

C
4-AMINO-p-TERPHENYL
MP 197-8°
5
4
3
2
A
B
C
A
B
ANILINE (75)
MP -6°
4-AMINOBIPHENYL
MP 53-4°
300
400
500

FIG 4

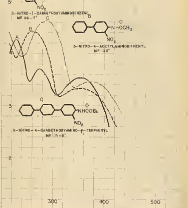

A
NHCOEt
NO2
2-NITRO-1-CARBETHOXYAMINOBENZENE
MP 56-7°
B
NHCOCH3
NO2
3-NITRO-4-ACETYLAMINOBIPHENYL
MP 132°
C
A
B
C
NHCOEt
NO2
3-NITRO-4-CARBETHOXYAMINO-p-TERPHENYL
MP 171-2°
5
4
3
2
300
400
500

FIG 5

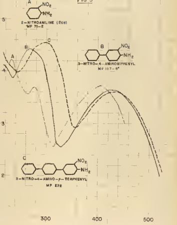

A
NO2
NH2
2-NITROANILINE (268)
MP 71-2
B
NO2
NH2
3-NITRO-4-AMINOBIPHENYL
MP 167-8°
C
NO2
NH2
3-NITRO-4-AMINO-p-TERPHENYL
MP 272
A
B
C
5
4
3
2
300
400
500

FIG 6

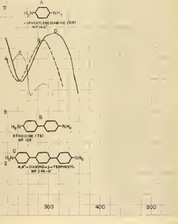

5
A
H₂N–C₆H₄–NH₂
p-PHENYLENEDIAMINE (218)
MP 140°
C
B
A
4
3
B
H₂N–C₆H₄–C₆H₄–NH₂
BENZIDINE (75)
MP 128
C
H₂N–C₆H₄–C₆H₄–C₆H₄–NH₂
2
4,4"-DIAMINO-p-TERPHENYL
MP 248-9°
300
400
500

FIG. 7

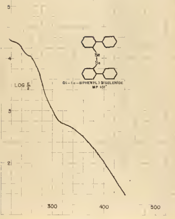

5
4
LOG $\frac{E}{2}$
3
2
300
400
500
Se
Se
DI-(o-BIPHENYL) DISELENIDE
MP 101°

FIG. 8

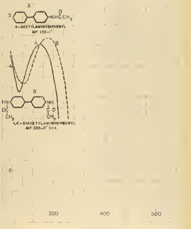

A
5
NHCCH₃
O
4-ACETYLAMINOBIPHENYL
MP 170-1°
A
B
4
B
HN
OC
CH₃
NH
C
O
CH₃
4,4'-DIACETYLAMINOBIPHENYL
MP 335-7° DEC
2
300
400
500

FIG 9

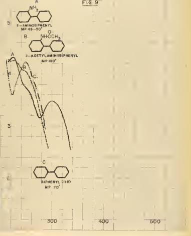

A
NH2
2-AMINOBIPHENYL
MP 49-50°
B
NHCCH3
O
2-ACETYLAMINOBIPHENYL
MP 120°
A
B
C.
5
4
3
2
C
BIPHENYL (118)
MP 70°
300
400
500

FIG 10

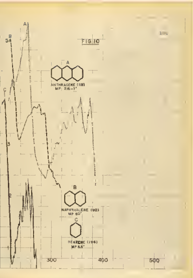

A
B
C
5
4
3
2
1
A
ANTHRACENE (88)
MP 216-7°
B
NAPHTHALENE (92)
MP 80°
C
BENZENE (196)
MP 5.5°
300
400
500

FIG II

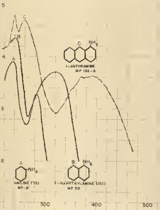

5
4
3
2
A
B
C
A
C
NH2
1-ANTHRAMINE
MP 122-3
A
NH2
ANILINE (75)
MP 6°
B
NH2
1-NAPHTHYLAMINE (152)
MP 50
300
400
500

FIG 12

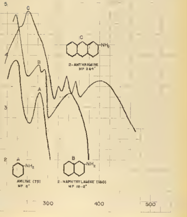

5
4
3
2
C
B
A
C
NH2
2-ANTHRAMINE
MP 244°
A
NH2
ANILINE (75)
MP 6°
B
NH2
2-NAPHTHYLAMINE (180)
MP 111-2°
300
400
500

FIG 13

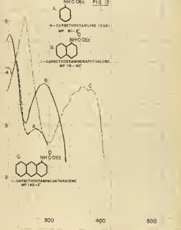

A.
NHCOEt
O
N-CARBETHOXYANILINE (268)
MP 51-2°
B.
NHCOEt
O
1-CARBETHOXYAMINONAPHTHALENE.
MP 78-80°
C.
NHCOEt
O
1-CARBETHOXYAMINOANTHRACENE
MP 140-1°
5
4
3
2
A
B
C
300
400
500

FIG 14

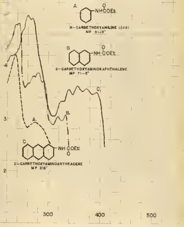

A
NHCOEt
O
N-CARBETHOXYANILINE (268)
MP 81-2°
B
NH-COEt
O
2-CARBETHOXYAMINONAPHTHALENE
MP 71-2°
C
NH COEt
O
2-CARBETHOXYAMINOANTHRACENE
MP 216°
A.
B.
C.
4
3
2
300
400
500

FIG 15

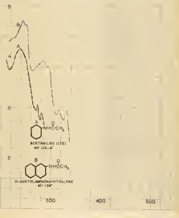

5
4
3
2
B
A
NHCCH₃
O
ACETANILIDE (173)
MP 113–4°
2-ACETYLAMINONAPHTHALENE
MP 134°
300
400
500

FIG 16

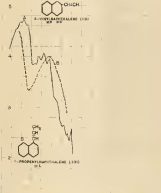

A
CH=CH
2-VINYLNAPHTHALENE (119)
MP 66
A
B
CH3
CH
CH
B
1-PROPENYLNAPHTHALENE (231)
OIL
5
4
3
2
300
400
500

Fig 17

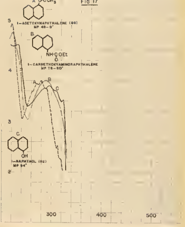

A
1-ACETOXYNAPHTHALENE (88)
MP 48-9°
B
NH-C(O)OEt
1-CARBETHOXYAMINONAPHTHALENE
MP 78-80°
C
OH
1-NAPHTHOL (82)
MP 94°
5
4
3
2
A
B
C
300
400
500

FIG 18

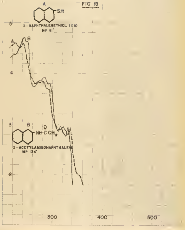

A
SH
2-NAPHTHALENETHIOL (119)
MP 81°
5
A
B
4
3
B
NH C CH3
O
2-ACETYLAMINONAPHTHALENE
MP 134°
2
300
400
500

FIG 19

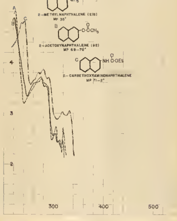

A
B
C
CH3
2-METHYLNAPHTHALENE (218)
MP 35°
O-C(=O)-CH3
2-ACETOXYNAPHTHALENE (92)
MP 69-70°
NH C(=O)-OEt
2-CARBETHOXYAMINONAPHTHALENE
MP 71-2°
4
3
2
300
400
500

FIG 20

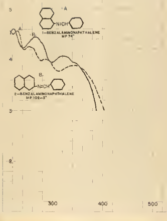

5
A
N=CH
1—BENZALAMINONAPHTHALENE
MP 74°
A
B
4
B.
N=CH
2—BENZALAMINONAPHTHALENE
MP 102-3°
3
2
300
400
500

FIG 21

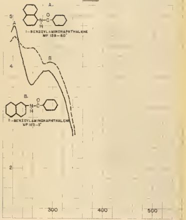

A.
1–BENZOYLAMINONAPHTHALENE
MP 159–60°
B.
2–BENZOYLAMINONAPHTHALENE
MP 162–3°
5
4
2
A
B
300
400
500

FIG 22

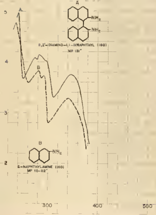

A
NH$_2$
NH$_2$
2,2'-DIAMINO-1,1'-DINAPHTHYL (160)
MP 191°
A
B
5
4
3
2
B
NH$_2$
2-NAPHTHYLAMINE (160)
MP 111-112°
300
400
500

FIG 23

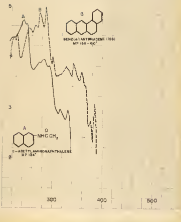

5
A
B
B
BENZ(a)ANTHRACENE (156)
MP 159–60°
4
3
A
O
NHC CH3
2-ACETYLAMINONAPHTHALENE
MP 134°
2
300
400
500

FIG 24

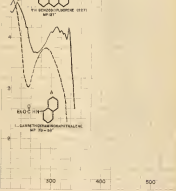

5
A
B.
B
7H BENZO(c)FLUORENE (227)
MP 127°
4
3
A
EtOC(O)HN
1-CARBETHOXYAMINONAPHTHALENE
MP 79-80°
2
300
400
500

FIG. 25

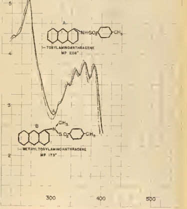

B
5
A.
NH-SO2
CH3
1-TOSYLAMINOANTHRACENE
MP 208°
4
3
A
B
N
CH3
SO2
CH3
1-METHYLTOSYLAMINOANTHRACENE
MP 173°
2
300
400
500

FIG 26

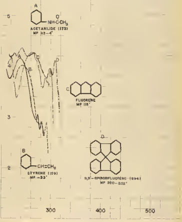

A
NH-C-CH3
O
ACETANILIDE (173)
MP 113-4°
C
FLUORENE
MP 115°
B
CH=CH2
STYRENE (159)
MP -33°
D
9,9'-SPIROBIFLUORENE (294)
MP 200-202°
5
4
3
2
300
400
500

FIG 27

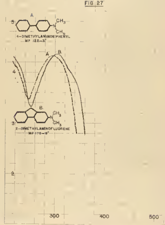
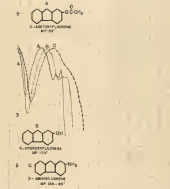

A
CH3
N
CH3
4–DIMETHYLAMINODIPHENYL
MP 122–3°
A
B
B.
CH3
N
CH3
2–DIMETHYLAMINOFLUORENE
MP 178–9°
5
4
3
2
300
400
500

FIG 28


A
2-ACETOXYFLUORENE
MP 129°
B
2-HYDROXYFLUORENE
MP 170°
C
2-AMINOFLUORENE
MP 128-30°
5
4
3
2
300
400
500

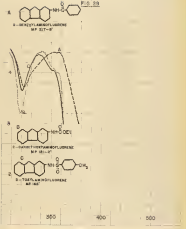


FIG 29

A
2-BENZOYLAMINOFLUORENE
MP 217-8°
NH-C
O
A
C
4
B
3
B
NHCOEt
O
2-CARBETHOXYAMINOFLUORENE
MP 121-2°
C
NH
S
O
O
CH3
2
2-TOSYLAMINOFLUORENE
MP 168°
300
400
500

FIG 30

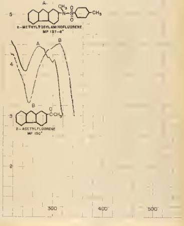

A
CH3
N
S
O
O
CH3
2-METHYLTOSYLAMINOFLUORENE
MP 137-8°
5
A
B
4
B
O
C-CH3
3
2-ACETYLFLUORENE
MP 130°
2
300
400
500

FIG 31

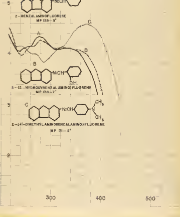

A
N=CH
2-BENZALAMINOFLUORENE
MP 158-9°
C
A
B
B
N=CH
OH
2-(2-HYDROXYBENZALAMINO) FLUORENE
MP 156-7°
C
N=CH
N
CH3
CH3
2-(4'-DIMETHYLAMINOBENZALAMINO) FLUORENE
MP 211-2°
5
4
3
2
300
400
500

FIG 32

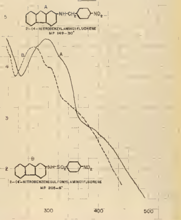

A
5
NH-CH$_2$ NO$_2$
2-(4-NITROBENZYLAMINO) FLUORENE
MP 149-50°
B
A
4
3
B
2
NH-SO$_2$ NO$_2$
2-(4'-NITROBENZENESULFONYLAMINO) FLUORENE
MP 205-6°
300
400
500

FIG 33

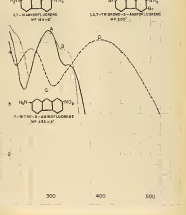

A
2,7-DIAMINOFLUORENE
MP 164-5°
B
1,3,7-TRIBROMO-2-AMINOFLUORENE
MP 200°
C
7-NITRO-2-AMINOFLUORENE
MP 232-3°
A
B
C
4
3
2
300
400
500

FIG 34

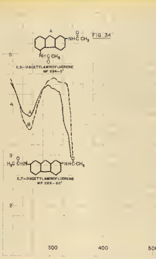

A
2,5-DIACETYLAMINOFLUORENE
MP 294-5°
2,7-DIACETYLAMINOFLUORENE
MP 289-90°
A
B
5
4
3
2
300
400
500

FIG 35

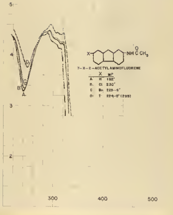

5
4
3
2
D
C
B
A
300
400
500
X
NH C CH$_3$
O
7-X-2-ACETYLAMINOFLUORENE
X MP
A. H 192°
B. Cl 230°
C Br 228-6°
D. I 224-5° (295)

FIG 36

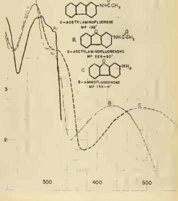

5
A
2-ACETYLAMINOFLUORENE
MP 192°
B
2-ACETYLAMINOFLUORENONE
MP 229-30°
C
2-AMINOFLUORENONE
MP 155-6°
A
3
B
C
2
300
400
500

FIG 37

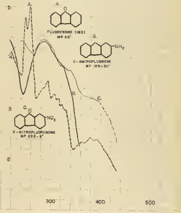

5
4
3
2
A.
A
FLUORENONE (160)
MP 83°
B.
NH$_2$
2-AMINOFLUORENE
MP 129–30°
B.
C.
C.
NO$_2$
2-NITROFLUORENONE
MP 222–3°
300
400
500

FIG 38

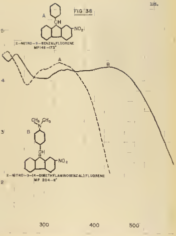

A
CH
NO2
2-NITRO-9-BENZALFLUORENE
MP146-173°
A
B
5
4
3
2
CH3 CH3
N
B
CH
NO2
2-NITRO-9-(4-DIMETHYLAMINOBENZAL) FLUORENE
MP 204-6°
300
400
500

FIG. 39

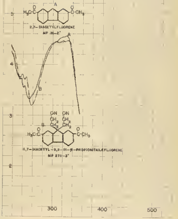

A
2,7-DIACETYLFLUORENE
MP 181-2°
A
B
5
4
3
2
B
2,7-DIACETYL-9,9-DI-β-PROPIONITRILEFLUORENE
MP 278-3°
300
400
500

FIG. 40

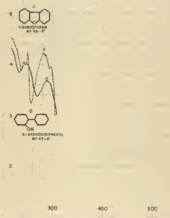

A
5
DIBENZOFURAN
MP 83–4°
A
4
B
B
3
OH
2-HYDROXYBIPHENYL
MP 57–8°
2
300
400
500

FIG 41

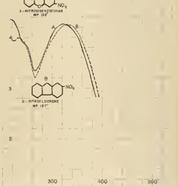
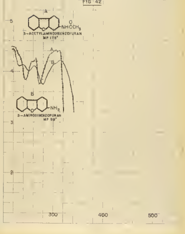

5
A
NO_2
3-NITRODIBENZOFURAN
MP 183°
A
B
4
B
NO_2
3
2-NITROFLUORENE
MP 157°
2
300
400
500

FIG 42


A
NHCCH$_3$
O
3-ACETYLAMINODIBENZOFURAN
MP 178°
A
B
B
NH$_2$
O
3-AMINODIBENZOFURAN
MP 98°
5
4
3
2
300
400
500

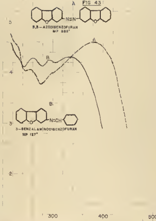


FIG 43

A
3,3–AZODIBENZOFURAN
MP 282°
N=N
O
A
B
B.
N=CH
3–BENZALAMINODIBENZOFURAN
MP 127°
5
4
3
2
300
400
500

FIG 44

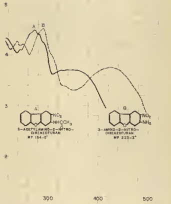

5
4
3
2
A
B
300
400
500
NO₂
NHCCH₃
O
3-ACETYLAMINO-2-NITRO-DIBENZOFURAN
MP 194-5°
NO₂
NH₂
3-AMINO-2-NITRO-DIBENZOFURAN
MP 222-3°

FIG 45

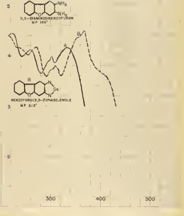

A
5
2,3-DIAMINODIBENZOFURAN
MP 166°
B
A
4
B
BENZOFURO(2,3-f)PIASELENOLE
MP 213°
3
2
300
400
500

FIG 46

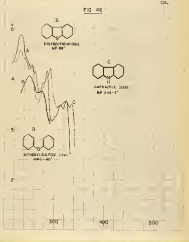

A
DIBENZOTHIOPHENE
MP 88°
C
CARBAZOLE (268)
MP 246–7°
B
DIPHENYLSULFIDE (174)
MP< -40°
ε
5
4
3
2
A
B
C
300
400
500

FIG. 47

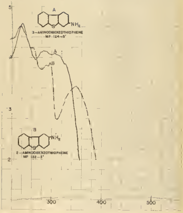

5
A
NH₂
S
3-AMINODIBENZOTHIOPHENE
MP 124–5°
A
B
3
B
NH₂
S
2-AMINODIBENZOTHIOPHENE
MP 132–3°
2
300
400
500

FIG 48

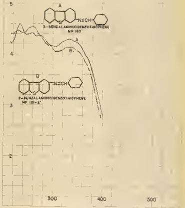

A
3-BENZALAMINODIBENZOTHIOPHENE
MP 160
A
B.
B
2-BENZALAMINODIBENZOTHIOPHENE
MP 181-2°
5
4
3
2
300
400
500

FIG 49

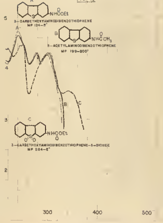

A
NHCOEt
3-CARBETHOXYAMINODIBENZOTHIOPHENE
MP 124-5°
B
NHCCH3
3-ACETYLAMINODIBENZOTHIOPHENE
MP 199-200°
C
NHCOEt
3-CARBETHOXYAMINODIBENZOTHIOPHENE-5-DIOXIDE
MP 224-5°
A
B
C
5
4
3
2
300
400
500

FIG 50

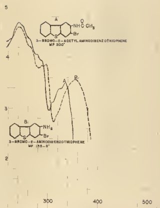

5
4
3
2
A
3-BROMO-2-ACETYLAMINODIBENZOTHIOPHENE
MP 200°
A
Br
B
3-BROMO-2-AMINODIBENZOTHIOPHENE
MP 155-8°
300
400
500

FIG. 51

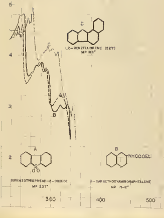

5
4
3
2
C
A
B
1,2-BENZFLUORENE (227)
MP 188°
DIBENZOTHIOPHENE-5-DIOXIDE
MP 237°
2-CARBETHOXYAMINONAPHTHALENE
MP 71-2°
NHCOOEt
S
O O
300
400
500

FIG 52

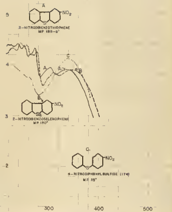

A
2-NITRODIBENZOTHIOPHENE
MP 186-8°
B
2-NITRODIBENZOSELENOPHENE
MP 180°
C
4-NITRODIPHENYLSULFIDE (174)
MP 55°
NO2
S
Se
A
B
C
5
4
3
2
300
400
500

FIG. 53

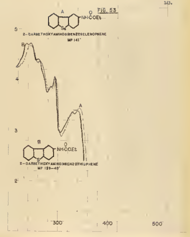

A
NH·C(=O)OEt
Se
2-CARBETHOXYAMINODIBENZOSELENOPHENE
MP 141°
B
NH·C(=O)OEt
S
2-CARBETHOXYAMINODIBENZOTHIOPHENE
MP 139-40°
5
4
3
2
300
400
500

FIG 54

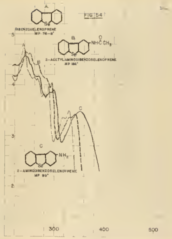

A
Se
DIBENZOSELENOPHENE
MP 76–8°
B
NH·C(=O)CH3
Se
2–ACETYLAMINODIBENZOSELENOPHENE
MP 182°
C
NH2
Se
2–AMINODIBENZOSELENOPHENE
MP 99°
A
B
C
5
4
3
2
300
400
500

FIG 55

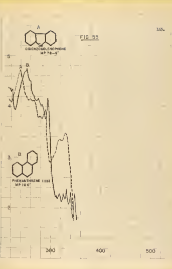

A
Se
DIBENZOSELENOPHENE
MP 76-8°
5
A
B
4
3
B
PHENANTHRENE (118)
MP 100°
2
300
400
500

FIG 56

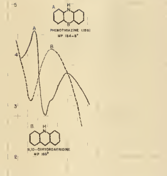

A
H
N
S
PHENOTHIAZINE (156)
MP 184-5°
5
A
B.
4
3
B.
H
N
9,10-DIHYDROACRIDINE
MP 169°
2
300
400
500

FIG 57

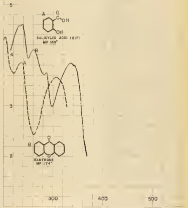

5
4
3
2
A
SALICYLIC ACID (217)
MP 159°
B
XANTHONE
MP 174°
A
B
300
400
500

FIG 58

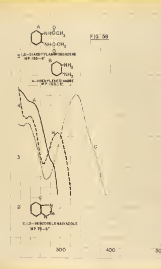

A
1,2-DIACETYLAMINOBENZENE
MP 185–6°
B
o-PHENYLENEDIAMINE
MP 103–4°
C
2,1,3-BENZOSELENADIAZOLE
MP 75–6°
5
4
3
2
A
B
C
300
400
500

FIG 59

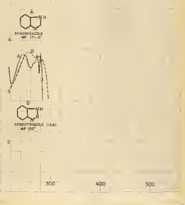

5
A
NH
N
BENZIMIDAZOLE
MP 171–2°
4
A
B
3
B
NH
N
N
BENZOTRIAZOLE (106)
MP 100°
2
300
400
500

FIG 60

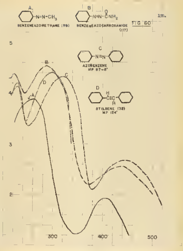

A
BENZENEAZOMETHANE (99)
B
BENZENEAZOCARBOXAMIDE (117)
C
AZOBENZENE
MP 67-8°
D
STILBENE (32)
MP 124°
A
B
C
D
5
4
3
2
300
400
500

FIG 61

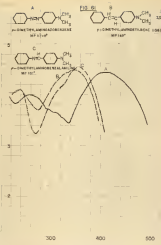

A
p-DIMETHYLAMINOAZOBENZENE
MP 117-8°
B
p-DIMETHYLAMINOSTILBENE (136)
MP 149°
C
p-DIMETHYLAMINOBENZALANILINE
MP 101°
5
3
2
A
B
C
300
400
500

FIG 62

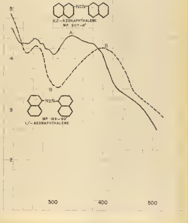

A
N=N
2,2'-AZONAPHTHALENE
MP 207-8°
A
B
B
N=N
MP 189-90°
1,1'-AZONAPHTHALENE
5
4
3
2
300
400
500

FIG. 63

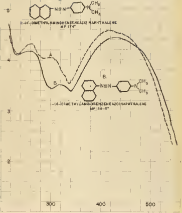

A
N=N
N
CH3
CH3
2-(4'-DIMETHYLAMINOBENZENEAZO) NAPHTHALENE
MP 174°
A
B.
B.
N=N
N
CH3
CH3
1-(4'-DIMETHYLAMINOBENZENEAZO) NAPHTHALENE
MP 134–5°
5
4
3
2
300
400
500

FIG 64

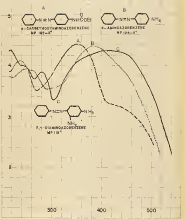

A
N=N
NHCOEt
O
4-CARBETHOXYAMINOAZOBENZENE
MP 162-3°
B
N=N
NH2
4-AMINOAZOBENZENE
MP 124-5°
C
N=N
NH2
NH2
2,4-DIAMINOAZOBENZENE
MP 118°
A
B
C
C
5
4
3
2
300
400
500

FIG. 65

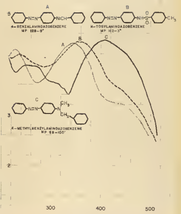

A
B
C
4-BENZALAMINOAZOBENZENE
MP 128–9°
N-TOSYLAMINOAZOBENZENE
MP 162–3°
4-METHYLBENZYLAMINOAZOBENZENE
MP 99–100°
N=N
N=CH
NH
S
O
O
CH_3
N
CH_3
CH_2
5
3
2
300
400
500

FIG. 66

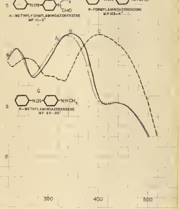

A
4-METHYLFORMYLAMINOAZOBENZENE
MP 111-2°
B
4-FORMYLAMINOAZOBENZENE
MP 183-4°
C
4-METHYLAMINOAZOBENZENE
MP 89-90°
A
B
C
5
4
3
2
300
400
500

FIG 67

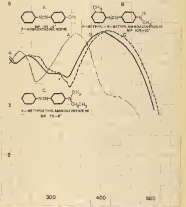

5
4
3
2
A
4-HYDROXYAZOBENZENE
MP 155-6°
B
CH$_3$
3'-METHYL-4-METHYLAMINOAZOBENZENE
MP 109-10°
C
CH$_3$
CH$_2$CH$_3$
4-METHYLETHYLAMINOAZOBENZENE
MP 72-3°
A
B
C
300
400
500

FIG 68

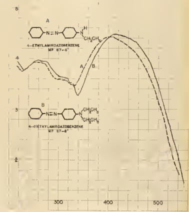

5
4
3
2
A
N=N
H
N
CH2CH3
4–ETHYLAMINOAZOBENZENE
MP 87–8°
A
B
B
N=N
N
CH2CH3
CH2CH3
4–DIETHYLAMINOAZOBENZENE
MP 87–8°
300
400
500

FIG. 69

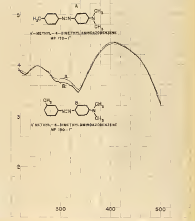

A
4'-METHYL-4-DIMETHYLAMINOAZOBENZENE
MP 170-1°
A
B
B
3'METHYL-4-DIMETHYLAMINOAZOBENZENE
MP 120-1°
5
4
3
2
300
400
500

FIG 70

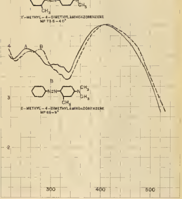

A
2'-METHYL-4-DIMETHYLAMINOAZOBENZENE
MP 73.5-4.0°
A
B
B
2-METHYL-4-DIMETHYLAMINOAZOBENZENE
MP 65-9°
5
4
3
2
300
400
500

FIG 71

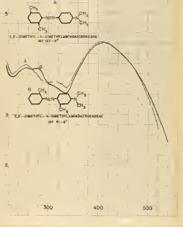

A
CH3
N=N
N
CH3
CH3
CH3
2,5'-DIMETHYL-4-DIMETHYLAMINOAZOBENZENE
MP 157-8°
A
B
B
CH3
N=N
N
CH3
CH3
CH3
2,2'-DIMETHYL-4-DIMETHYLAMINOAZOBENZENE
MP 81-2°
5
3
2
300
400
500

FIG. 72

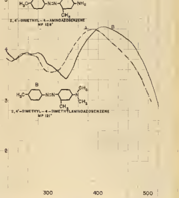

A
H3C–N=N–NH2
CH3
2,4'-DIMETHYL-4-AMINOAZOBENZENE
MP 128°
A
B
B
H3C–N=N–N(CH3)2
CH3
2,4'-DIMETHYL-4-DIMETHYLAMINOAZOBENZENE
MP 121°
5
4
3
2
300
400
500

FIG 73

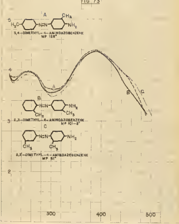
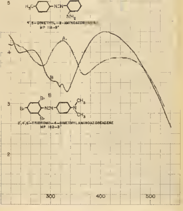

A
3,4-DIMETHYL-4-AMINOAZOBENZENE
MP 128°
B
2,3-DIMETHYL-4-AMINOAZOBENZENE
MP 101-2°
C
2,3'-DIMETHYL-4-AMINOAZOBENZENE
MP 81°
A
B
C
5
4
3
2
300
400
500

FIG 74


A
H3C
N=N
CH3
NH2
4',5-DIMETHYL-2-AMINOAZOB
MP 118-9°
5
4
3
2
A.
B
B
Br
Br
Br
N=N
N
CH3
CH3
2',4',6'-TRIBROMO-4-DIMETHYLAMINOAZOBENZENE
MP 162-3°
300
400
500

C. Determination of the Dissociation Constant

1. Theory. The basic dissociation constants of many slightly soluble compounds have not been determined because of the experimental difficulties in applying the usual titration or electromotive-force methods to very low concentrations. This difficulty has been taken care of by Flexser *et al* (114), who have calculated dissociation constants of weak acids and bases from the ultraviolet spectra. Further details on the method have been given by Sager and Siewers (262).

The calculation of the basic strength is made as follows - - if the solute exists in two forms, free base, B, and salt, BH^+, so that the total concentration, C_m, is equal to the sum of the concentrations of the two forms,

$$C_m = C_B + C_{BH^+}$$

and if, as seems generally to be true, each substance absorbs independently of the presence of the other, then

$$E = (k_B C_B + k_{BH^+} C_{BH^+})l$$

where E is the optical density, k_B is the specific extinction of the free base at a molar concentration, C_B, k_{BH^+} is the specific extinction of the salt at a molar concentration, C_{BH^+}, and l is the length of the path of light through the solution. It follows from

$$k_m = \frac{E}{C_m l}$$

that $$k_m C_m l = (k_B C_B + k_{BH^+} C_{BH^+})l$$

On substitution

$$k_m (C_B + C_{BH^+}) = (k_B C_B + k_{BH^+} C_{BH^+})$$

When this equation is rearranged

$$k_m C_{BH^+} - k_{BH^+} C_{BH^+} = k_B C_B - k_m C_B$$

So

$$\frac{C_{BH^+}}{C_B} = \frac{k_B - k_m}{k_m - k_{BH^+}}$$

As k = E/Cl, and C and l are constant,

$$\frac{C_{BH^+}}{C_B} = \frac{E_B - E_m}{E_m - E_{BH^+}}$$

The dissociation of a salt in aqueous solution may be represented as

$$BH^+ \rightleftharpoons B + H^+$$

so that the dissociation constant, Ka, is given by

$$Ka = \frac{(B)(H^+)}{(BH^+)} \frac{fB}{f_{BH^+}}$$

From this we obtain the equation

$$pKa = pH + \log \frac{(BH^+)}{(B)} + \log \frac{f_{BH^+}}{f_B}$$

The ratio f_{BH^+}/f_B of the activity coefficients of ion and base is very close to unity, since the dye concentrations are 5×10^{-5}M.

On this basis

$$\log \frac{f_{BH^+}}{f_B} = 0$$

and finally we obtain the equation to determine the dissociation constant with the help of the spectrophotometer.

$$pKa = pH_m + \log \frac{E_B - E_m}{E_m - E_{BH^+}}$$

Because concentrations as low as $5x10^{-5}M$ cannot be obtained for many organic compounds in water solution, 50% alcohol has come into favor as the solvent of choice in spectrophotometric pK determinations (3,4,5,93,289). The calculations are the same as for pure water except that pKa cannot be converted to pK_B with any degree of accuracy. Since the pKa values are determined in a medium of low ionic strength, they should differ little from the values at zero ionic strength (256).

2. Procedure. The dye (10^{-4} moles) was accurately weighed, quantitatively transferred to a 100 ml. volumetric flask and diluted to the mark with filtered commercial 95% ethyl alcohol. Five ml. of this $10^{-3}M$ solution was transferred to a second 100 ml. volumetric flask. Fifty ml. of the appropriate aqueous acid or alkaline solution was added. The dye solution was then diluted to the mark with the 95% alcohol. Thus, all final dye solutions were $5x10^{-5}M$. In order to avoid errors caused by changes in the temperature of the solution, the addition of the last few ml. was delayed until the solution had attained room temperature, 26 ± 1° .

All absorption spectra were measured by means of a Beckman quartz photoelectric spectrophotometer, Model DU, at a temperature of 26 ± 1°. The blank in all cases consisted of distilled water as there was no discernable differences spectrally between distilled water and 50% alcoholic acid or alkaline solutions. Corex cells were used for the wave length range of 3200 to 6000 A. The instrument was balanced at an initial wave length of 3200 A. and an initial slit width of 0.4 mm. For measurement at other wave lengths the 'sensitivity' was not changed, while the slit width was varied for each wave length measurement to balance the instrument. The readings of optical density were taken at intervals of 40 A. in the pK determination region and at the maxima, otherwise intervals of 50 A. were used.

As an examination of the spectral curves of the azo dyes in solutions of different pH revealed that the strong acid curves (1N, 2N and 4N HCl) did not pass through the isobiestic points, it was apparent a new substance, BH_2^{++}, was present which had been absent in weak acid solutions. This was the reason the maxima at around 5200 A. displayed considerable increase in intensity for the 2.0 N HCl solution as compared to the 1.0 N HCl solution. So the curves in this region could not be used in the pK determinations. On the other hand the strong acid solutions of the dye had the same spectral curve in the minima region near the 4000 A. whether they passed through the isobiestic points or not, while the curves of the basic solution and the weak acid solutions of the dye passed through the same isobiestic points. On this basis all curves in this region indicated that only B and BH^+ were present, so the curves between the isobiestic points at approximately 3500 to 4800 A. were used for the pK determinations.

3. Calculations. In the determination of the pKa of the azo dyes the derived formula was used.

$$pKa = pHa + \log \frac{E_B - E_m}{E_m - E_{BH^+}}$$

E_B is the optical density of a 50% alcoholic 0.01 N sodium hydroxide solution of the dye, E_m is the optical density of the 50% alcoholic solution of the dye at a pHa with a numerical value close to the pKa of the dye, and E_{BH^+} is the optical density of a 50% alcoholic 1 N hydrochloric acid solution.

For example, the pKa of 4-methylethylaminoazobenzene was calculated at a wave length of 3900 in the following manner. E_B was 0.816, E_m(0.005 N HCl) was 0.446 and E_{BH^+} was 0.019. From Fig. 75 it can be seen that 0.005 N HCl has a pH of 2.64. With these values we can calculate the pKa.

$$pKa = 2.64 + \log \frac{0.816 - 0.446}{0.446 - 0.019}$$

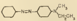
$$= 2.64 - 0.06 = 2.58$$

Tables VII and VIII show some of the typical values obtained from the curves.

4. Plotting of Spectral Curves. For each azo dye, spectral curves were obtained in 0.01 N HCl, 0.01 N NaOH, 1 N HCl and 2 N HCl. As a check the 0.01 N HCl curve was repeated on a new solution or new curves were obtained at one or two more intermediate pH values. For the sake of clarity some of the curves have been left out, for example, the 2 N HCl curve. In all the spectral curves the optical

TABLE VII

THE pKa OF 4-METHYLETHYLAMINOAZOBENZENE AT pH 2.64

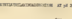


	pH = 2.64*				
	E_B	E_m	E_{BH^+}	$\log \frac{E_B - E_m}{E_m - E_{BH^+}}$	pKa
390	0.816	0.446	0.019	-0.062	2.58
395	0.908	0.498	0.023	-0.064	2.58
400	1.00	0.550	0.025	-0.067	2.57
404	1.05	0.583	0.030	-0.073	2.57
408	1.10	0.611	0.035	-0.062	2.58
412	1.144	0.636	0.044	-0.066	2.57
416	1.17	0.653	0.052	-0.065	2.57
420	1.184	0.663	0.062	-0.062	2.58
424	1.195	0.674	0.074	-0.061	2.58
428	1.195	0.678	0.086	-0.059	2.58
432	1.205	0.690	0.108	-0.053	2.59
436	1.197	0.698	0.129	-0.057	2.58
440	1.191	0.708	0.151	-0.062	2.58
450	1.154	0.723	0.225	-0.063	2.58

* A Beckman pH meter and glass electrode were used for all pH measurements.

TABLE VIII

THE pKa OF 4-METHYLETHYLAMINOAZOBENZENE AT pH 2.34

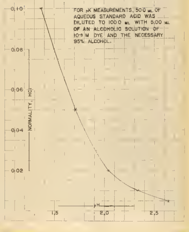


	E_B	pH = 2.34 E_m	E_{BH^+}	$\log \frac{E_B - E_m}{E_m - E_{BH^+}}$	pKa
390	0.816	0.299	0.019	0.266	2.61
395	0.908	0.335	0.023	0.264	2.60
400	1.00	0.368	0.025	0.265	2.61
404	1.05	0.393	0.030	0.258	2.60
408	1.10	0.417	0.035	0.252	2.59
412	1.144	0.435	0.044	0.258	2.60
416	1.17	0.448	0.052	0.261	2.60
420	1.184	0.457	0.062	0.265	2.61
424	1.195	0.467	0.074	0.268	2.61
428	1.195	0.478	0.086	0.262	2.60
432	1.205	0.490	0.108	0.272	2.61
440	1.191	0.517	0.151	0.264	2.60
450	1.154	0.558	0.225	0.253	2.59
460	1.064	0.592	0.330	0.256	2.60

density, E, was plotted against the wave length in millimicrons. The coordinate scale was selected so that in the abscissa 20 mμ equaled 1 cm. and in the ordinate 0.1 of the intensity unit equaled 1 cm. On this basis the spectral curves of the dyes could be compared by superimposition. For each spectral curve the strength of acidity or alkalinity was denoted by the type of line used for the curve, e.g.

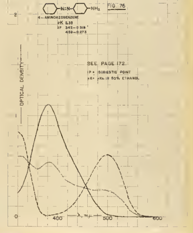
0.01 N NaOH	――――――――――――
0.01 N HCl	....
1.0 N HCl	— — — — — — —
0.10 N HCl	— . — . — . — .
0.05 N HCl	— .. — .. — .. —
0.02 N HCl	— ... — ... — ...
0.008 N HCl	— — . — — . — —
0.007 N HCl	.. — — .. — — ..
0.005 N HCl	... — — ... — —
0.002 N HCl	.... — — .... — —

FIG. 75 '50%' ETHANOL



FOR pK MEASUREMENTS, 50.0 ML OF AQUEOUS STANDARD ACID WAS DILUTED TO 100.0 ML WITH 5.00 ML OF AN ALCOHOLIC SOLUTION OF 10^{-3} M DYE AND THE NECESSARY 95% ALCOHOL.

FIG. 76



pK 2.38
IP 342—0.318
459—0.273

SEE PAGE 172.

IP = ISOBIESTIC POINT
pK = pKa IN 50% ETHANOL

FIG. 77

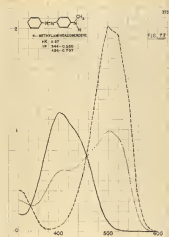

CH3
N=N
N
H
4-METHYLAMINOAZOBENZENE
pK 2.37
IP 344-0.250
456-0.737
2
1
0
400
500
600

FIG. 78.

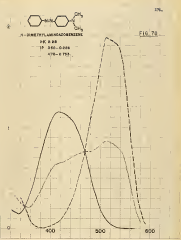
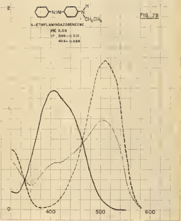
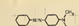

2
CH3
N=N
N
CH3
4-DIMETHYLAMINOAZOBENZENE
pK 2.28
IP 350–0.226
470–0.753
1
0
400
500
600

FIG. 79

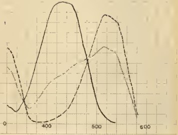

2
4-ETHYLAMINOAZOBENZENE
pK 2.58
1P 348–0.301
464–0.664
1
0
400
500
600

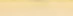


4-METHYLETHYLAMINOAZOBENZENE

pK 2,58
IP 384-0.219
480-0.643

FIG. 80

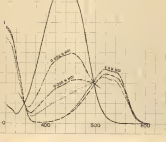




2

4-DIETHYLAMINOAZOBENZENE
pK 3.09
IP 387-0.191
496-0.413

FIG 81



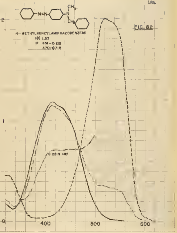
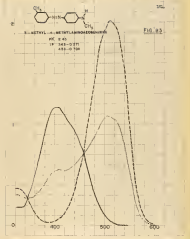


FIG. 82

4-METHYLBENZYLAMINOAZOBENZENE
pK 1.87
P 351 – 0.212
470 – 0.718
CH3
N=N
N
CH2
2
1
0
0.05 N HCl
400
500
600

FIG. 83


CH3
N=N
H
N
CH3
3-METHYL-4-METHYLAMINOAZOBENZENE
PK 2.43
IP 343-0.271
488-0.708
2
1
0
400
500
600

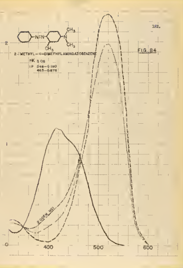
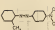

2
2-METHYL-4-DIMETHYLAMINOAZOBENZENE
pK 3.08
IP 348-0.180
463-0.876
1
0.005N HCl
0
400
500
600

FIG. 84

2

FIG. 85



2'-METHYL-4-DIMETHYLAMINOAZOBENZENE

pK 2.04

IP 360—0.367
492—0.240

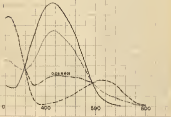
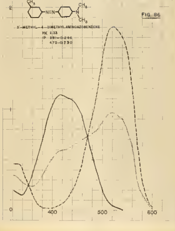
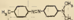


FIG 86


2
3'-METHYL-4-DIMETHYLAMINOAZOBENZENE
PK 2.33
IP 381-0.246
470-0.730
0
400
500
600

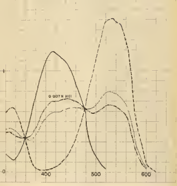
2



4'-METHYL-4-DIMETHYLAMINOAZOBENZENE

pK 2.38
IP 358 - 0.338
479 - 0.624

FIG. 87



2

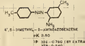
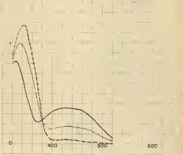


FIG. 88

4',5-DIMETHYL-2-AMINOAZOBENZENE

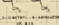
pK 2.80

IP 316-0.760 (BY EXTRAPOLATION)
378-0.240

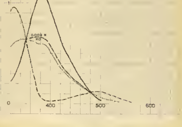


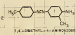
187.

FIG. 89



2,3-DIMETHYL-4-AMINOAZOBENZENE
pK 3.29
IP 351-0.640
489-0.094



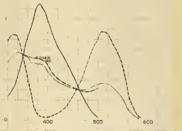


3,4'-DIMETHYL-4-AMINOAZOBENZENE

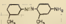
pK 2.35

IP 351–0.650
462–0.302

FIG. 90



2

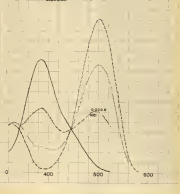


2,3'-DIMETHYL-4-AMINOAZOBENZENE

PK 2·92

IP 543–0.466
446–0.431

FIG. 91



2

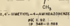
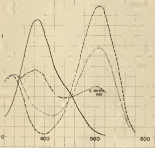


FIG. 92

2,4'-DIMETHYL-4-AMINOAZOBENZENE

pK 2.92
IP 348-0.688
483-0.404



2

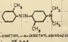
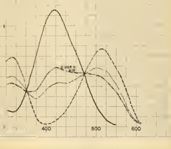


FIG. 93

2,2'-DIMETHYL-4-DIMETHYLAMINOAZOBENZENE

pK 2.64

IP 360-0.354
430-0.332



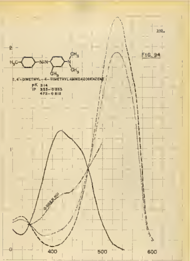
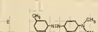


FIG. 94

2,4'-DIMETHYL-4-DIMETHYLAMINOAZOBENZENE

pK 3.14
IP 355-0.283
472-0.812

FIG 95



2',5'-DIMETHYL-4-DIMETHYLAMINOAZOBENZENE

pK 2.0
IP 361-0.420
493-0.283

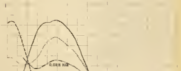
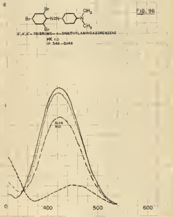


FIG. 96



2',4',6'-TRIBROMO-4-DIMETHYLAMINOAZOBENZENE

pK 1.0

IP 346-0348

V

ANCILLARY PROBLEMS

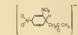
A. Differentiating Color Test for Fluorene Derivatives

In 1929 Maitland and Tucker (193) reacted fluorene with acetone in the presence of potassium hydroxide to obtain a compound which analyzed for $C_{19}H_{20}O$ and melted at 76-78°. Eight years later, France, Maitland and Tucker (115) proved the structure of this compound to be methyl β-9-fluorenyl-β-methyl-n-propyl ketone. The authors reported that a dark blue-green color was formed in this reaction. Fluorene was also condensed with mesityl oxide in the presence of potassium hydroxide to give the same compound in poorer yield. Again a green color was obtained. This color could have been due to reaction on the fluorene molecule or to impurities. It was found in our laboratory that the formation of color was due to interaction on the fluorene molecule, for many fluorene compounds reacted in a similar fashion.

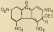
It is probable that the 9- position in fluorene is the position attacked, for electron-attracting groups in the 2- position activate the 9- position and do not hinder the color test, while electron-repelling groups in the 2- position which deactivate the 9- position do not give the blue-green color.

The reaction of fluorene compounds with acetone in the presence of alkali arranges fluorene derivatives into three distinct groups. In the first group are included fluorene itself, and fluorene derivatives with no electron-attracting group in the 2- position. These compounds give a blue-green color in the test. In the second group are fluorene derivatives with an electron-repelling group in the 2-position. These compounds do not give any vivid color. In Table IX the blue-green is shown to be specific for the fluorene molecule, while in Table X the two groups of fluorene derivatives are compared. Acidic hydrogen interferes with the test for compounds in the first group, as shown in Table XI.

In the third group shown in Table XII are the fluorenone derivatives and other compounds with electron-attracting groups in the m-positions. These compounds give a violet color with acetone in the presence of alkali. Canback (67, 68, 69, 70, 71, 72, 73, 74) has made a vigorous study of the reaction in alkaline solution between m-dinitrobenzenes and compounds containing activated methylene groups. He postulates that in the reaction between m-dinitrobenzene and acetone in alkaline solution a violet quinonoid-like compound is formed,



where ''the bond between the benzene nucleus and the acetone ion must be regarded as a bond between a dipole and an ion with many resonance forms" (69). The mono- and dinitrofluorenones, like the m-dinitrobenzenes, give a negative reaction in alkaline alcohol. The striking fact that 2,4,5,7-tetranitrofluorenone forms a violet color in alkaline alcohol is not surprising, for so does s-trinitrobenzene. This can be attributed to the fact that both molecules have the requisite order of activity necessary for reaction with the ethoxide ion to form complexes of the type (see 69):



Canback (73) has postulated that it is possible for the m-dinitrobenzenes to react with a series of anions, such as $CH_3COCH_2^-$, CH_3O^-, OH^-, NH_2^-, CN^-, etc., to give colors of decreasing intensity dependent on the stability of the quinonoid complex. The similarity of the formulas and the similarity of the color reactions with alkaline acetone and alkaline alcohol of the 2-nitrofluorenones and m-dinitrobenzenes is definite evidence that the violet color is due in all cases to one general overall reaction. This is the reaction in alkaline solution between a compound containing an aromatic ring with 2 or 3 electron-attracting groups in meta positions and a suitably activated anion to give a violet to purple adduct of uncertain structure. In this respect, compounds containing a methylene group, activated by an electron-attracting group, form suitably activated anions in alkaline solution.

The fact that 2-acetylfluorenone gives a violet color in alkaline alcohol is not surprising. The molecule has 2 electron-attracting groups in the m position and also has an activated methylene group.

Thus, the molecule can react with itself in alkaline solution to form the violet complex.

In Table XIII it can be seen that 2-nitrofluorenone, like m-dinitrobenzene (73), reacts with ketones containing an active methylene group. The sensitivity of the reaction is shown by the fact that cyclopentanone gives a discernible color reaction at a dilution of 1:10,000 with an alkaline saturated alcoholic solution of 2-nitrofluorenone.

The test procedure for Tables IX, X and XI is as follows: into a warm solution or suspension of 0.2 g. of the compound in 2 ml. of acetone was added 0.5 g. of powdered sodium hydroxide. The mixture was refluxed gently over a steam bath for 5 minutes. Any change in color was noted.

The test procedure for Table XII is as follows: to a solution or suspension of 0.1 g. of the compound in 2 ml. of acetone or alcohol was added 0.5 g. of powdered sodium hydroxide. The color was read in 2 minutes.

The test procedure for Table XIII is as follows: to a solution or suspension of 0.1 g. of the ketone in 2 ml. of a saturated alcoholic solution of 2-nitrofluorenone was added 0.5 g. powdered sodium hydroxide. The color was read within several minutes.

TABLE IX

FLUORENE AND ANALOGS

Compound	Color
Fluorene	blue-green
9,9'-Difluorene	blue-green
Biphenyl	colorless
p-Terphenyl	colorless
9,10-Dihydroacridine	light yellow
Anthrone	red
Dibenzofuran	colorless
Dibenzothiophene	colorless
Dibenzoselenophene	colorless

TABLE X

FLUORENE DERIVATIVES

Compound	Color
Electron-attracting	
2-Chlorofluorene	blue-green
2-Nitrofluorene	blue-green
2-Nitro-7-chlorofluorene	blue-green
2-Benzoylfluorene	blue-green
2-Acetylfluorene	green
2-Benzalaminofluorene	green
2-Cinnamalaminofluorene	green
2-(4'-Dimethylaminobenzalamino)fluorene	green
2,5-Dinitrofluorene	blue-green
2,7-Dinitrofluorene	blue-green
2-Nitro-7-benzoylfluorene	blue-green
2,7-Diacetylfluorene	blue-green
2,2'-Difluorenylketone	blue-green
Electron-repelling	
2-Fluorenediazonium chloride	brownish
2-Aminofluorene	yellow
2-Acetoxyfluorene	yellow
2-Dimethylaminofluorene	light pink
2-Acetylaminofluorene	light pink
2-Benzoylaminofluorene	yellow
2-(o-Carboxybenzoylamino)fluorene	pink
2-Tosylaminofluorene	white ppt.
2,7-Diaminofluorene	brownish
Phenyl-2-fluorenylcarbinol	pinkish
Phenyl-2-fluorenyl-S-phenylthiocarbinol	yellowish
2-(4'-Dimethylaminophenylazo)fluorene	brownish
2-(4'-Nitrobenzenesulfonylamino)fluorene	red-brown

TABLE XI

INFLUENCE OF
SUBSTITUENTS WITH ACTIVE HYDROGEN

Compound	Color
2-(o-Carboxybenzoyl)fluorene	white ppt.
Fluorene-2-sulfonic acid	pink
2-(4'-Hydroxybenzalamino)fluorene	yellow ppt.
2-(2',4'-Dihydroxybenzalamino)fluorene	yellow ppt.

TABLE XII

COMPOUNDS WITH TWO META ELECTRON-ATTRACTING GROUPS

Compound	Color with acetone	Color with alcohol
2-Nitrofluorenone	violet	yellow
2,5-Dinitrofluorenone	violet	light-green
2,7-Dinitrofluorenone	violet	light-green
2,4,7-Trinitrofluorenone	violet	green-brown
2,4,5,7-Tetranitrofluorenone	violet	dark violet
2-Acetylfluorenone	purple (on warming)	violet
2-(2'-Piperidinoethyl)-fluorenonecarboxylate	violet-brown *	—
2-(2'-Dimethylaminoethyl)-fluorenonecarboxylate	brown-red *	—
2-Nitrodibenzothiophene-5-dioxide	light violet	—
2-Nitroanthraquinone	red violet	—
2,4-Dinitrophenylhydrazine	purple	—
m-Dinitrobenzene	blue-violet	colorless

* Here again the presence of acidic hydrogen interferes.

TABLE XIII

KETONES

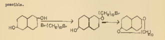
Compound	Color
Acetone	violet
Methyl ethyl ketone	violet
Methyl isobutyl ketone	violet
Methyl n-amyl ketone	violet
Acetophenone	violet
Cyclohexanone	violet
Cyclopentanone	violet
2-Acetylnaphthalene	violet
2-Acetylfluorene	violet
2,7-Diacetylfluorene	violet

8. Diels-Alder Reactions of Maleimide

Determination of the spatial configuration of naturally occurring and synthetic steroids is a problem which requires much further research before a satisfactory method for ascertaining the structure of these compounds can be developed. The methods in use suffer from such serious limitations that thus far it is possible to portray only the probable formulas of many steroids.

When two carboxyl, hydroxyl or amine groups are cis and adjacent they readily form a new ring, thus proving their relationship. Some similar method is needed when these groups are more distant from each other.

Luttringhaus (291) has shown that this type of reaction is possible.



In 1940 Butz, Gaddis, Butz and Davis (61) heated 2,5-dimethyl-1-5-hexadiene-3-yne with maleic anhydride and obtained 1,5-dimethyl-2,3,4,6,7,8-hexahydronaphthalene-3,4,7,8-tetracarboxylic acid dianhydride. Similarly, Joshel, Butz and Feldman (162) by reacting 1,1'-dicyclohexenylacetylene with maleic anhydride obtained tetradecahydrochrysene-5,6,11,12-tetracarboxylic acid dianhydride. It was not determined whether the anhydride groups were cis or trans.

It is apparent that if a nonactivated dienophile could be reacted with a dienyne, a ring closure could probably be affected between the two active groups of the adduct and a polymethylene derivative, forming a Luttringhaus type compound.

Much time was spent in unsuccessful attempts to react such dienophiles as acrolein, acrylonitrile or crotonic acid with dienynes. In this respect Butz and Joshel (62) were unable to add ethylene to the dienynes.

It was finally decided to investigate maleimide as a dienophile in the Diels-Alder reaction with dienynes. The dipotassium salt of the adduct could then, conceivably, be reacted with a polymethylene dihalide to form a large ring compound. If the two dicarboximides were cis, fewer methylene groups would be necessary in the formation of the large ring than in the case where these groups were trans to each other.

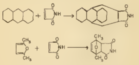
In the formation of 1,1'-dicyclohexenylacetylene an insoluble by-product was obtained. Analyses and molecular weight determinations indicated the compound to be $C_{16}H_{24}O_2$. As the 1-cyclohexenylacetylene type of compound is a known by-product in the Nazarov synthesis, it is believed that $C_{16}H_{24}O_2$ may be a dimer of 1-cyclohexenylacetylene.

Tust and Guyard (291) attempted to prepare the dienyne from 2,5-diphenyl-3-hexyne-2,5-diol. They found that the diol was very sensitive to acids; only resins were obtained. We obtained 2,5-diphenyl-1,5-hexadiene-3-yne in poor yield by dehydration in an inert atmosphere.

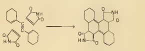
Maleimide was prepared by a modification of the procedure of Plancher and Cattadori (235). Blomquist and Winslow (36) and Harvey

(144), were successful in bringing about its reaction with cyclopentadiene. A comparison of maleic anhydride and maleimide seemed to favor maleimide as the more active dienophile. For example, anthracene refluxed with maleic anhydride in xylene for fifteen minutes gave a quantitative yield of the adduct as reported by Clar (80), while the reaction of anthracene with maleimide in boiling xylene gave a violent exothermic reaction after two minutes of refluxing.

When maleic anhydride was reacted with 2,5-dimethylfuran in dry ether at 0°, an adduct started to separate after four to six hours standing (60). For the same reaction with maleimide, crystals started to separate immediately. In all cases, however, a practically quantitative yield of adduct was obtained.



This evidence of the reactivity of maleimide suggested that it might be suitable for our purpose. It is of course less desirable than a monobasic compound. The acidic hydrogen attached to the nitrogen, however, offers no unequivocal method of linking the opposite sides together. In conformity with our predictions, 1,1'-dicyclohexenylacetylene and maleimide when heated in xylene for 7 hours gave a good yield of tetradecahydrochrysene-5,6,11,12-tetracarbondiimide. Only one stereo-isomer could be isolated.



Unfortunately so much time and material had been expended in unsuccessful attempts to condense monobasic dienophiles that time did not permit the author to carry out experiments to join the nitrogens and bridge the molecule. The great reactivity of maleimide should make it very useful in the Diels-Alder reaction.

C. A Spot Test For Unhindered Aromatic Amines and Aromatic Sulfonyl Chlorides

In a study of the physical properties of the carcinogenic amines a color test has been developed for unhindered aromatic amines. When an aromatic amine and an aromatic sulfonyl chloride are dissolved in pyridine, a sulfonamide is obtained. A by-product of unknown constitution is also formed. Dependent on the type of amine used, the color of this by-product can be yellow to violet. In the formation of this color pyridine seems to be necessary, for if acetic acid, dimethylaniline, quinoline, acetone or benzene are substituted for pyridine, no coloration is observed. In Table XIV are listed the color reactions of many unhindered aromatic amines. For comparison Table XV lists aliphatic and hindered amines that give a yellow to colorless fleck.

This color reaction can also be used in testing for aromatic sulfonyl chlorides. For example, in Table XVI are listed seven aromatic sulfonyl chlorides which gave a violet fleck with a solution of 2-aminofluorene in pyridine. It is apparent that this color reaction is a general test for unhindered primary amines, aromatic sulfonyl chlorides and ortho substitution in aromatic primary amines.

From an examination of the tables the following conclusions can be drawn.

(1) Unhindered aromatic primary amines and aromatic sulfonyl chlorides react in pyridine solution to give orange to violet colored by-products.

(2) Alkylanilines, 'hindered' aromatic amines and aliphatic amines give a colorless to yellow fleck in the reaction test.

(3) With an increase of steric hindrance the color obtained is shifted towards the ultraviolet.

(4) An increase in the conjugation of the hetero base has a B effect on the color.

In testing for an unhindered aromatic amine, Tables XIV and XV, the following procedure was used. A small drop or a crystal of the amine was placed on filter paper. A small drop of the reagent was added. The reagent drop was allowed to spread. The reaction spot was finally dried over a warm hot plate and the color was read. Unhindered aromatic primary amines gave a red to violet fleck. Other aromatic amines and aliphatic amines gave a colorless to yellow fleck. The reagent consisted of a fresh solution of 1 g. of p-nitrobenzenesulfonyl chloride dissolved in 25 ml. of pyridide. This reagent was not stable, for after several days standing it would not give the test reactions.

In testing for aromatic sulfonyl chlorides, Table XVI, the same procedure was used except that the reagent consisted of 1 g. of 2-aminofluorene in 25 ml. of pyridine.

TABLE XIV

UNHINDERED AROMATIC PRIMARY AMINES

Compound	Color
Aniline	orange
m-Hydroxyaniline	pink
m-Aminobenzoic acid	orange
m-Toluidine	red-orange
p-Toluidine	red
o-Phenylenediamine	red
2,4-Diaminotoluene	red
1-Naphthylamine	red
2-Naphthylamine	violet
4-Aminobiphenyl	red-violet
2-Aminofluorene	violet
3-Aminodibenzofuran	violet
3-Aminodibenzothiophene	violet
4-Amino-p-terphenyl	violet
2-Aminodibenzothiophene	violet
2-Aminodibenzoselenophene	violet
2-Amino-7-nitrofluorene	brown-violet
2,5-Diaminofluorene	violet
2,7-Diaminofluorene	violet
Benzidine	violet
4,4''-Diamino-p-terphenyl	violet
p-Phenylenediamine	violet
4-Aminoazobenzene	red-brown

TABLE XV

AMINES GIVING A YELLOW TO COLORLESS FLECK

Compound	Color
Piperidine	light yellow
2-Aminopyridine	yellow
4,6-Dimethyl-2-aminopyridine	yellow
2,4,6-Tribromoaniline	colorless
2-Amino-4'-nitrobiphenyl	bright yellow
2-Aminobiphenyl	light yellow
Hexamethylenediamine	faint yellow
Methylaniline	yellow
o-Toluidine	faint yellow
Methylethylaniline	faint yellow
Diethylaniline	faint yellow
Di-n-propylaniline	faint yellow
Methylbenzylaniline	faint yellow
1,3,7-Tribromo-2-aminofluorene	colorless
2-Amino-3-bromodibenzothiophene	orange

TABLE XVI

SULFONYL CHLORIDES WHICH REACT WITH 2-AMINOFLUORENE IN PYRIDINE TO GIVE THE COLOR TEST

Compound	Color
p-Acetaminobenzenesulfenyl chloride	red-violet
p-Toluenesulfenyl chloride	violet
p-Nitrobenzenesulfenyl chloride	violet
m-Nitrobenzenesulfenyl chloride	violet
2-Nitro-7-fluorenesulfenyl chloride	red-violet
2,7-Fluorenedisulfenyl chloride	violet
2,4-Dimethylbenzenesulfenyl chloride	violet

VI

RESULTS AND DISCUSSION

A. Syntheses

Although we were concerned mainly with the spectral and basic properties of the known carcinogenic and non-carcinogenic amines, it was necessary to synthesise a fairly large number of new compounds. Many series of compounds had members missing and so had to be completed. Other compounds had to be prepared which, on the basis of our results, could be predicted to be carcinogenic. Many new derivatives were prepared for the purpose of furthering an understanding of the spectral properties of the carcinogenic amines. Several groups of compounds were prepared for the express purpose of studying the K region sequence spectrally. This sequence will be discussed more fully in section VIC5 of this paper.

The structures of several new compounds of dubious constitution were established. In this respect, the nitration of dibenzoselenophene gave a yellow compound whose analysis indicated the presence of one nitro group in the molecule. As dibenzothiophene, dibenzoselenophene and carbazole are iso-pi-electronic, they are consequently isospectral, i.e., their ultraviolet absorption spectra are similar, Figs. 46 and 55. On this basis any group of analogous derivatives of these three compounds should also be somewhat isospectral in the same solvent. Comparison of the spectral curves of 1-nitrocarbazole and 3-nitrocarbazole (268) with 2-nitrodibenzothiophene and nitrodibenzoselenophene, Fig. 52, brought out the fact that 3-nitrocarbazole, 2-nitrodibenzothiophene and nitrodibenzoselenophene were isospectral. In addition, it was found that while 3-aminodibenzothiophene, Fig. 47, was not isospectral with aminodibenzoselenophene, Fig. 54, the 2-isomer, Fig. 47, was isospectral. Similarly 3-carbethoxyaminodibenzothiophene, Fig. 48, was not isospectral with carbethoxyaminodibenzoselenophene, Fig. 53, while the 2-isomer, Fig. 53, was isospectral. If nitration of dibenzoselenophene would have taken place in the 3-position, the spectra of the nitro compound would have borne no resemblance to the parent compound or to 2-nitrodibenzothiophene. The spectral curve would have resembled the curve of 4-nitrobiphenyl as do the curves of 3-nitrodibenzofuran and 2-nitrofluorene, Fig. 41. If nitration had taken place in the 1-position, the nitro compound and the derived compounds (especially the carbethoxyamine) would have shown a definite steric effect as compared to 2-nitrodibenzothiophene and its derivatives instead of resembling them spectrally. Some of these facts are shown in Table XVII. Thus, the proof is conclusive that nitration of dibenzoselenophene gives 2-nitrodibenzoselenophene.

The nitration of 4-carbethoxyamino-p-terphenyl gave glistening orange-yellow crystals that analyzed for a mononitro-4-carbethoxyamino-p-terphenyl. The absorption spectra of this compound is compared with 3-nitro-4-acetylaminobiphenyl and 2-nitro-1-carbethoxyaminobenzene in Fig. 4. Comparison of this sequence and the o-nitroamine sequence in Fig. 5 with the p-amino homologue sequence of Fig. 3

TABLE XVII

ISO-PI-ELECTRONIC DIBENZOTHIOPHENE AND DIBENZOSELENOPHENE DERIVATIVES

Compound	Band I	Band II	Band III
	Wave length maxima in mu.		
Dibenzothiophene	324	285	233
Dibenzoselenophene	328	287	238
2-Aminodibenzothiophene	348	296	236
2-Aminodibenzoselenophene	351	298	241
2-Carbethoxyaminodibenzothiophene	340	293	237 246
2-Carbethoxyaminodibenzoselenophene	343	295	241 245

shows clearly that a new low energy band has been formed in the nitro compounds. This band is of only moderate intensity because of the ortho effect. In p-nitroamines this band is of higher intensity and lower wave length, e.g., 4-nitroaniline, λ 372, $\log \varepsilon$ 4.18 (268), 2-amino-7-nitrofluorene, λ 400, $\log \varepsilon$ 4.25 (Fig. 33) and 4'-nitroxenylamine, λ 378, $\log \varepsilon$ 4.20 (275). From a study of these homologous sequences, it is apparent that the new compound is 3-nitro-4-carbethoxyamino-p-terphenyl.

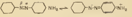
The reaction of acrylonitrile with 2,7-diacetylfluorene gave colorless crystals melting at 272-3°. The assignment of structure for the new compound is based on the following facts. If the acetyl groups in 2,7-diacetylfluorene had been attacked, it is probable that 3 or 6 moles of acrylonitrile would have been added to the molecule, for an examination of the literature reveals that in the known cyanoethylation reactions, the acetyl group always adds three moles of acrylonitrile (57). However, analysis has shown that under the conditions of the experiment 2,7-diacetylfluorene adds only 2 moles of acrylonitrile, as does fluorene (56). The reaction of fluorene with acrylonitrile gives bis-9,9-(2-cyanoethyl)fluorene. As the two acetyl groups in 2,7-diacetylfluorene would cause the methylene group in the 9-position to be much more active than it is in fluorene, it is only reasonable to believe that the 9-position was attacked in the diacetyl compound. On this basis the structure tentatively assigned the new compound is 2,7-diacetyl-9,9-di-β-propionitrilefluorene.

. Dissociation Constants of the Azo Dyes

1. Introduction. As pi basicity is a common characteristic of all the aromatic carcinogens while n basicity is a specific property of the carcinogenic amines, the question arises whether the amine group is necessary for carcinogenesis. If the amine group is necessary because of its hetero basicity, then a study of the dissociation constants of some specific group of carcinogenic and non-carcinogenic amines should bring out a relationship between carcinogenesis and hetero basicity. Many azo dyes are known to form malignant tumors in the liver of the rat. These dyes are fairly specific in their action. For this reason the n basicity of the aminoazo dyes has been studied. Because the rat was the specific animal tested with the azo dyes and the liver was the specific organ attacked, we can assume that the chemical environment of the host was approximately constant in all the animal experimentation. Thus, the only factors that are not constant are the chemical and physical properties of these azo dyes. For a thorough understanding of the dyes such properties as geometry, catabolism, lipophilic-hydrophilic distribution, pi and n electronic transitions and pi basicity should be investigated. It is apparent that no one worker could attempt to study all these properties in a short span of time. We can assume that in the aminoazo carcinogens most of these properties depend to some extent on the amino group and its basicity. On this basis we can proceed with our discussion of hetero basicity.

In azobenzene we have two N atoms in close proximity. As is pyrimidine, pyridazine and pyrazine, the effect of the resultant n electronic interactions is twofold. (a) Azobenzene is a very weak base(5). (b) It's absorption spectra shows a well developed low energy n band of fairly high intensity. Thus, in n electronic interactions weak basicity is apparently associated with the presence of n bands.

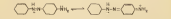
In the aminoazobenzenes the resonance effect causes the beta N to acquire a high electron density. Consequently an acid will be



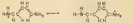
preferably attracted to the beta N. This has been stated to occur with sulfur dioxide (185) and palladium dicyanide (109). In the reaction with a proton it is evident that if the proton attached itself to the amino N, the consequent pi→sigma transition would form a molecule, iso-pi-electronic with azobenzene. As a result the salt and azobenzene would be isospectral. Actually this is not so, for the spectral curves of azobenzene, Fig. 60, and 4-dimethylaminoazobenzene in 1 N HCl, Fig. 78, are dissimilar. Therefore the proton attaches itself to either the alpha or beta N. On the basis of the resonance effect it is believed that the beta N attracts the proton because of its increased electron density. As a methyl group in the 2-position of butter yellow increases its basicity and a methyl group in the 2' position decreases the basicity, the beta N must be the basic N of the molecule, for just as in o-toluidine, the

2'-methyl group has an inductive effect on the basic beta N causing a decrease in basicity (Table XVIII). Here for the first time definite experimental evidence is presented to show that the beta N, and not the alpha N, is the basic N of the molecule.

2. Substituent effects on basicity. Despite the fact that in the aminoazobenzenes we have a phenyl amine with a strong negative group in the para position and the base weakening effect of the two adjacent N atoms, the compounds have a moderate base strength. The base strengthening pi n'↔n pi' transition is the evident explanation of this anomaly.



The powerful effect of this transition can be seen in the following compound (272), which bears quite a resemblance to 4-aminoazobenzene.



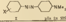
pK= 12

It is evident that this base is stronger than would have otherwise been predicted. In Tables XVIII and XIX the basicities of aminoazo dyes are compared with analogous aniline derivatives. The comparative effects of diverse groups on the 2 molecules show a surprising similarity.

The electron attracting effect of the benzyl group in benzyl-

TABLE XVIII

pKa COMPARISONS OF ANILINE AND AZOBENZENE DERIVATIVES

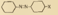


X		pKa in H_2O	X	pKa in 50% ETOH	
4-OMe	(138)	5.29	4'-OMe	2.35	(256)
4-Me	(138)	5.07	4'-Me	2.36	*
3-Me	(138)	4.69	3'-Me	2.33	
H	(138)	4.58	H	2.28	**
2-Me	(138)	4.39	2'-Me	2.04	
4-Cl	(138)	4.00	4'-Cl	2.00	(256)
4-NO_2	(179)	1.0	4'-NO_2	1.81	(256)
2,4,6-Br_3	(137)	abt.-0.8	2',4',6'-Br_3	1.0	

* Rogers et al (256) obtained 2.31.
** Rogers *et al* obtained 2.27.

TABLE XIX

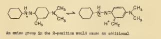
pKa OF ANILINE AND AZOBENZENE DERIVATIVES IN 50% ALCOHOL



X	(93)	
NEt_2	5.85	3.08
NMeEt	—	2.58
NHEt	—	2.58
NHMe	—	2.37
NH_2	4.26	2.35
NMe_2	4.21	2.28
N-Me$CH_2\phi$	—	1.97

amine, pKa 9.30, as compared to methylamine, pKa 10.64 (183), is also found in 4-methylbenzylaminoazobenzene as compared to 4-dimethylamino-azobenzene, Table XIX. The well known base strengthening effect of N-substituted ethyl groups occurs also in the aminoazobenzene series, Table XIX.

The base strengthening effect of a methyl group in the 2-position of the 4-aminoazobenzene derivatives, Table XX, is caused by a resonance effect similar to the pi n'↔n pi' transition.



An amine group in the 2-position would cause an additional pi n'↔n pi' transition. In this respect 2,4-diaminoazobenzene has an approximate pKa of 4.6 as compared to a pKa of 2.35 in 4-amino-azobenzene. Essentially this resonance effect causes an increase in electron density of the beta N.

The base weakening effect of a methyl group in the 2' position of the 4-aminoazobenzene compounds is evidently caused by an ortho effect consisting mainly of the inductive effect of the methyl group on the beta N. A methyl group in the 3' or 4' position of the molecule causes a slight increase in basicity. The effect of a methyl group in the 3-position of a 4-aminoazobenzene is practically negligible. On the other hand in 4-dimethylaminoazobenzene, a 3-methyl group would cause steric hindrance. This would tend to cause a base strengthening of the dimethylamino group, but at the same time a

TABLE XX

pKa OF 2 and 2'-SUBSTITUTED AMINOAZOBENZENE DERIVATIVES

Compound	pKa 50% ETOH
2-Methyl-4-dimethylaminoazobenzene	3.08
2,3'-Dimethyl-4-aminoazobenzene	2.92
2,4'-Dimethyl-4-aminoazobenzene	2.92
2,4'-Dimethyl-4-dimethylaminoazobenzene	3.14
2,2'-Dimethyl-4-dimethylaminoazobenzene	2.64
4-Dimethylaminoazobenzene	2.28
4-Aminoazobenzene	2.35
2'-Methyl-4-dimethylaminoazobenzene	2.04
2',5'-Dimethyl-4-dimethylaminoazobenzene	2.0
2',4',6'-Tribromo-4-dimethylaminoazobenzene	1.0

decrease in the pi n'↔ n pi' transition would cause a base weakening effect in the beta N. On this basis one could expect a decrease in pKa.

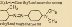
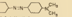
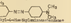
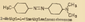
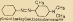
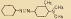
The explanations advanced in this section of the paper are based on the many analogous examples found in the literature as discussed in Part XIIA.

3. Correlation. The dissociation constants of approximately twenty four azo dyes were determined. The two most active carcinogens were found to have a pKa of 2.33 to 2.43, three with a carcinogenic index of 6 had a pKa of 2.26 to 2.58 and four others with a carcinogenic index less than 6 had a range in pKa of 2.04 to 2.64. For 4-methylformylaminoazobenzene, carcinogenic index of 1-2, the pKa could not be determined because in weak acid solution this compound was hydrolyzed to the more potent 4-methylaminoazobenzene.

The effect of basicity on the carcinogenic index of a homogeneous group of compounds is graphically illustrated in Table XXI. In Fig. 97 the correlation between the carcinogenic index and the pKa of the 4-aminoazobenzenes is shown. At a pKa of approximately 2.4 the dyes were either very active or relatively inactive carcinogens. The pKa ranged from 2 to 3.2 for the carcinogenic azo dyes. The graph definitely suggests that while the pKa is probably of primary importance, other physical and chemical properties will have to be studied to account for the anomalous position of compounds 21, 24, 38, 44 and 46. In this respect the geometry of the molecule is of extreme importance. From the evidence available in bio-organic and organic

TABLE XXI

MONOMETHYL-4-DIMETHYLAMINOAZOBE[illegible]

Compound	pKa	Carc. Index
2'-Methyl-4-dimethylaminoazobenzene 	2.04	2-3
4-Dimethylaminoazobenzene 	2.28	6
3'-Methyl-4-dimethylaminoazobenzene 	2.33	11
4'-Methyl-4-dimethylaminoazobenzene*	2.36	1
		
2-Methyl-4-dimethylaminoazobenzene 	3.08	1
3-Methyl-4-dimethylaminoazobenzene** 	—	0

* In the case of this molecule, substitution in the important extended para position has decreased the activity, because the optimum length of the molecule necessary for maximum activity has been exceeded.

** The dimethylamine group is bent out of the plane of the molecule by the adjacent methyl group. Thus the resonance with the remainder of the molecule is curtailed to a large extent causing the molecule to lose its activity.

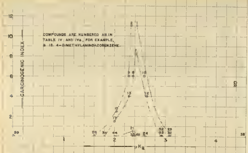


FIG. 97 CORRELATION OF THE CARCINOGENIC INDEX (OF THE 4-AMINOAZOBENZENE DERIVATIVES) WITH THE pKa.

chemistry, it is very probable that the electronic and geometrical structure of a potential aromatic carcinogen determines the amount of approachability and chemical activity necessary for a 'fit' between the carcinogen and the chemical and biological structures upon which the carcinogen acts. The interference with approachability may be the reason why substituted alkyl groups larger than a methyl group usually cause a carcinogen to lose all or some of its activity. For example, a methyl group on an amine N would tend to lie in the plane of a molecule while the methyl end of an ethyl group would stick out above the plane. The carcinogenic inactivity of twelve dyes in Table IVa can be traced to the presence of acidic hydrogen in the molecule. It is very probable that a difficultly hydrolyzable acyl group or a properly placed negative group can, by lowering the pKa, destroy the activity of a potential carcinogen. We have shown that the parent hydrocarbons of the carcinogenic amines are capable of a planar position. The absorption spectra has shown that the free electrons on the amine group interact very readily with the pi systems of these parent hydrocarbons. On this basis the carcinogenic amines are planar molecules. 4-Methylaminoazobenzene, carcinogenic index 6, is an example of a planar amine. When this compound is substituted with a 3-methyl group, there is a partial pi → π transition. The movement of the methylamine group is restricted. This ortho effect causes a drop in C.I. to 1. For 4-dimethylaminoazobenzene the free electrons on the nitrogen of the dimethylamine group can interact freely with the pi electrons of the remainder of the molecule and so these electrons are pi electrons. If a methyl group is placed in an ortho position to the dimethylamino group, the latter is pushed out of the plane of the molecule. Consequently the free electrons on the hetero group cannot interact as freely with the pi electrons of the molecule. As a result there is a partial pi → a transition and the new molecule takes on some of the properties of azobenzene and loses its carcinogenic activity. In this respect the powerful steric effect of a 3-methyl group in the butter yellow series has been spectrally proven recently (11).

Let us examine the compounds in Tables IV and IVa more fully as to the relation between their geometry and activity. We will confine ourselves to a discussion of the length, width and thickness of the molecule as it affects the activity. The Van der Waals radii of some of the atoms and groups are reported to be H, 1.2 A.; F, 1.35 A.; Cl, 1.80 A.; Br, 1.95 A.; CH_3, 2.0 A. and I, 2.15 A. (230). Compounds 1 through 6 have an activity of 10-12. Apparently for maximum activity a compound has to have the following qualifications. (a) Either it has to have a length equivalent to that of 4'-fluoroDAB. (b) Or its width has to fall in the range of 3'-fluoroDAB or 3'-methylDAB. (c) Its pKa in 50% alcohol should be approximately 2.3-2.5. (d) The molecule has to be planar. It is probable that the first five compounds are in the neighborhood of the optimum pKa, while compound 6 might be less. In this respect compound 7 would have approximately the same basicity as 6, but would not have the optimum length. If compounds 1 through 4 are considered as the reference compounds, the following changes will decrease the activity:

(a) Increasing or decreasing the length of the molecule, Table XXII.

(b) Increasing or decreasing the width, Table XXIII.

(c) Increasing or decreasing the pKa from the optimum range of 2.3 to 2.5, Fig. 97.

(d) Increasing the thickness or non-planarity, Table XXIV.

(e) The addition of a group which contains acidic hydrogen will also tend to decrease the activity, Table IVa, compounds # 22, 28, 29, 30, 31, 41, 42, 57 and 58.

Using these different effects the activity or inactivity of the majority of the azo dyes are capable of explanation. For example, the majority of the exceptions in Fig. 97 can be explained. Compounds 21 and 24 have the requisite basicity and the minimum width but are apparently too long for a proper 'fit', while compound 38 has a desirable basicity and a minimum width, but is evidently not long enough. On the other hand the inactivity of 44 is probably caused by its low basicity and excess width. Finally the author would like to emphasize that this theory based on the pKa and molecular dimensions of the azo dyes may prove to have some exceptions for it is evident that other properties may also prove to be of some importance.

4. Relationship of the azo carcinogens to other carcinogenic amines. Since the pKa's were determined by the author in 50% alcohol, they are comparable with one another but will differ from the values observed in water. As the pKa's of butter yellow and methyl orange

TABLE XXII

THE EFFECT OF INCREASING LENGTH ON THE CARCINOGENICITY OF SOME AZO DYES

Compound	C. I.
4-Aminoazobenzene	0
4-Dimethylaminoazobenzene (DAB)	6
4'-FluoroDAB	10
4'-ChloroDAB	1-2
4'-MethylDAB	0-1
4-Diethylaminoazobenzene *	0
4-Di-n-propylaminoazobenzene *	0
4-Di-n-butylaminoazobenzene *	0
4-Di-n-amylaminoazobenzene *	0

* In these compounds there is also an increased thickness as compared to DAB.

TABLE XXIII

THE EFFECT OF
INCREASING WIDTH ON CARCINOGENICITY

Compound	C. I.
DAB	6
3'-FluoroDAB	10
3'-ChloroDAB *	5-6
3'-MethylDAB	10-12
3'-NitroDAB *	5
3'-EthoxyDAB	1
2',5'-DimethylDAB	0
3',5'-DimethylDAB	0

* These compounds are predicted to be the least basic azo dyes in this table. Although they have an ideal width, the lowered basicity would affect their carcinogenicity.

TABLE XXIV

THE EFFECT OF INCREASING
THICKNESS OR NON-PLANARITY ON CARCINOGENICITY

Compound	C. I.
DAB	6
4-Methylethylaminoazobenzene	6
3-Methyl-4-methylaminoazobenzene	1
3-Methyl-DAB	0
4-Methylbenzylaminoazobenzene	0

are one unit higher in water than in 50% alcohol (256), we can reasonably assume that the pKa's of the carcinogenic azo dyes range from 3 to 5 in water. On this basis Fig. 98 indicates the striking fact that the pKa's of all the other measured carcinogenic amines, Table XXV, fall within the range of 3 to 5 also.

It is probable that the activity of all the carcinogenic amines depends upon the pKa and the molecular geometry to a large extent. The section on pi basicity will discuss this particular aspect more fully.

5. Predictions. It is evident that when a new theory is presented, its limitations and ramifications need to be thoroughly investigated. The length, width, thickness and pKa of the azo dyes could be varied to form all types of combinations. Three of the variables could be kept approximately constant while the fourth one could be altered gradually. For example, if the width was the variable being studied, the following series of compounds of increasing width and predicted decreasing activity could be constructed, such as 3'-fluoroDAB, 3'-chloroDAB, 3'-bromoDAB and 3'-iodoDAB, or 2-fluoroDAB, 2-chloroDAB, 2-bromoDAB and 2-iodoDAB. The following series of increasing width and increasing thickness with a predicted decrease in activity could be studied -- 3-fluoro-4-methylaminoazobenzene, 3-chloro-4-methylaminoazobenzene, 3-bromo-4-methylaminoazobenzene and 3-iodo-4-methylaminoazobenzene. An alkyl substituted series of increasing width and a predicted decreasing activity worthy of study is the following series of compounds -- 3'-methylDAB, 3'-ethylDAB,

TABLE XXV

THE pKa IN WATER OF CARCINOGENIC AMINES (136)

Compound	pKa	Carcinogenicity
Aniline *	4.58	0
2-Naphthylamine	4.11	+
2-Anthramine	app. 3-4 **	+
2-Aminonaphthacene	3 **	?
2-Aminobiphenyl ***	3.78	+
4-Aminobiphenyl	4.27	+
Benzidine (183)	4.97	+

* The inactivity of this molecule is probably due to its small area.

** These are approximate values based on the fact that the pi basicity increases in the benzene naphthalene series. Consequently hetero basicity would decrease to some limiting value.

*** In a personal communication to Dr. F. E. Ray, Dr. H. P. Morris has stated that the acetyl derivative is carcinogenic.

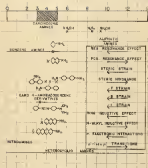


FIG. 98 THE SPECTRUM OF BASICITY

3'-propylDAB and 3'-butylDAB. The following compounds of increasing length are predicted to have an increase in activity for the second and third members followed by a decrease in activity -- 3'-methyl-4-aminoazobenzene, 3'-methyl-4-methylaminoazobenzene, 3'-methylDAB, 3'-methyl-4-methylethylaminoazobenzene, 3'-methyl-4-methylpropylaminoazobenzene and 3'-methyl-4-methylbutylaminoazobenzene. The dyes, 4'-fluoro-2-methylDAB, 4'-chloro-2-methylDAB, 4'-bromo-2-methylDAB and 4'-iodo-2-methylDAB, are predicted to decrease in basicity and activity. Many other series of derivatives could be constructed, but the examples given adequately illustrate the types of compounds that need to be studied.

On approximately the same basis the pKa and geometry of the other carcinogenic amines should also be studied. For example, the limiting length for the family of carcinogenic amines should be determined. In this respect 4,4"-diamino-p-terphenyl, which can be predicted to have a pKa in value of approximately 4 to 5, and 4-amino-p-terphenyl, predicted pKa <4, should be checked for carcinogenic activity. On the basis of the facts there is a good possibility that these amines (or some of their derivatives) will be found to be carcinogenic.

It is very probable from our work that the pKa's of 2,7-diaminofluorene, 2-aminofluorene, 3-aminodibenzofuran, 3-aminodibenzothiophene and 2-aminoanthracene will fall within the optimum range. It is desirable that the pKa's of the p-amines of the d region sequence should be investigated. The change in pKa from 4-aminobiphenyl to

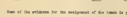
2-aminophenanthrene should give some information as to the inductive effect and the n→pi transition effect of the changing K region.

X = H_2, CH_2, O, S, Se, NH, CH=CH

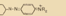
C. Spectral Studies

The study of the absorption spectra is of primary importance in relating the different groups of aromatic carcinogens to each other and in understanding the aromatic structure of these carcinogens.

1. Azobenzene derivatives. Azobenzene, Fig. 60, has three spectral bands, 229, 317 and 441 mμ. The band at 229 mμ is primarily a benzenoid pi band. The band at 317 mμ is a quinonoid pi band, for which some of the contributing structures are



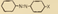
Some of the evidence for the assignment of the bands is presented in Table XXVI. Substitution of a p-amino grouping in azobenzene has a moderate B effect on the benzenoid pi band, but the effect on the quinonoid band is much more pronounced. The contribution from the p-quinonoid forms, such as



is increased. Consequently the p-quinonoid band shows a larger B effect. The assignment of bands is a very difficult problem. It is believed that a study of isospectral substances will facilitate such an assignment. In this paper is introduced the concept that isospectral molecules do not owe their spectral similarity to accident, but are similar

TABLE XXVI

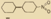
THE CHANGE IN WAVE LENGTH AS COMPARED TO AZOBENZENE



X	Benzenoid Pi Band mμ	Quinonoid Pi Band mμ	n Band mμ
Diethylamine	31	98	—
Methylethylamine	28	94	—
Dimethylamine	27	91	—
Methylbenzylamine	24	89	—
Ethylamine	27	88	—
Methylamino	26	86	—
Amino	21	66	—
Formylmethylamine	3	61	-12
Benzalamino	7	40	- 9
Hydroxy	6	32	-10
Formylamine	7	30	- 7
Carbethoxyamine	7	35	-27
Tosylamine	-2	29	-11
Polarization		Longitudinal	

because there is a definite electronic relationship common to the whole family. In a study of the 2 pi bands of isospectral stilbene and azobenzene, several miscellaneous compounds were found to be isospectral with azobenzene, Fig. 60, and stilbene, Fig. 60. For example, 4-nitrobiphenyl (V) (94), benzal alkyl ketones (IV) (155) and 1-phenylbutadiene (III) (45) were found to be closely isospectral with stilbene (I), while 2-nitrofluorene (VII), Fig. 41, and p-dimethylaminobenzaldehyde (VI) (305) were closely isospectral with the 2 pi bands of azobenzene. On this basis all the compounds are fairly isospectral to each other.

I

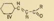

V

II

VI

III

VII


IV

Examination of the structures shows that the 2 pi bands of azobenzene and stilbene seem to be characteristic of the conjugation of a benzene ring with two properly situated groups (para or in a chain) each containing a pair of easily available free electrons. In the case of a para substituted benzene, one group has to be electronegative and the other has to be electropositive for the new compound to be isospectral with stilbene or azobenzene. The substitution of a double bond for one of the benzene rings of stilbene has little effect in changing the shape of the curve. This isospectral effect caused by the appropriate substitution of a double bond for a benzene ring will also be shown for fluorene in section VIC3. Examples of this isospectral effect can be seen in the comparison of (a) acetanilide, Fig. 15, with naphthalene, Fig. 10, (b) 2-acetylaminonaphthalene with benz(a)anthracene, Fig. 23, (c) 1-carbethoxyaminonaphthalene with 3,4-benzfluorene, Fig. 24, and 2-carbethoxyaminonaphthalene with 1,2-benzfluorene, Fig. 51. From an examination of the structures of the isospectral molecules, it can be predicted that p-nitroacetanilide and p-nitrostilbene will also be isospectral with the 2 pi bands of azobenzene.

The addition of a p-amino group to the isospectral pair, 2-nitrofluorene and azobenzene gives 2-nitro-7-aminofluorene, Fig. 33, and 4-aminoazobenzene, Fig. 64, which are isospectral with each other. Similarly, it can be predicted that 4-aminostilbene will be found to be isospectral with 4'-nitro-4-aminobiphenyl.

In half the examples shown in Table XXVI, the x band has been thoroughly enveloped by the quinonoid pi band. In the other examples where the B effect on the quinonoid band was not as large, the n band shows the -B effect of a p-amino grouping. This -B effect has been shown previously for N-benzeneazopiperidine (58), pyrazine and pyridazine (139). Further evidence for the correct assignment of the bands is seen in Fig. 60. Stilbene is isospectral with azobenzene except for the fact it does not have an n band. Compounds, such as azomethane (140), 1-benzeneazo-1-phenylcyclohexane (91), 1-azo-bis-1-arylalkanes (83), cinnoline (145), pyridazine (139), 2,2'-azopyridine (184) and the azonaphthalenes, Fig. 62, have low intensity, low energy n bands also. Further evidence that the low intensity band of azobenzene is an n band is the fact that it can be obliterated by an n→pi or n→sigma transition through the addition of oxygen to form an azoxy compound (107), the addition of a 4-trifluoromethyl group (276) or solution in concentrated sulfuric acid (58).

2. Naphthalene sequence. For the sake of clarity and ease of discussing the three main spectral bands of this homologue sequence are designated as I, II and III, Table XXVII. The assignment of the bands is based primarily on the work of Badger (17), Jones (152,158,159,160, 161), Klevens (170,172) and Clar (79,80,81).

With increasing chain length, band II moves to the red with greater jumps than do the other bands. In the case of anthracene, band II has caught up with band I and has enveloped it, Table XXVII. A substituent, whose free electrons interact readily with the parent pi system, causes a B effect. When the substituent is in the 1-position

TABLE XXVII

THE BENZENE-NAPHTHALENE SEQUENCE

Compound	I	II	III
	Wave length maxima in mu *		
Benzene	254	208	183
N-Carbethoxyaniline	274	236	?
Naphthalene	302	276	220
1-Carbethoxyaminonaphthalene	—	290	222
2-Carbethoxyaminonaphthalene	332	280	243
Anthracene	—	356	255
1-Carbethoxyaminoanthracene	—	362	253
2-Carbethoxyaminoanthracene	—	354	260
Polarization	Longitudinal	Transverse	Longitudinal

* Average was taken of all the vibrational levels of each band except when some vibrational level was more prominent than the others.

of naphthalene or anthracene, band II is displaced toward the visible; when the substituent is in the 2-position, bands I and III are so displaced. In 1-carbethoxyaminonaphthalene the II band has covered band I. The same phenomena is seen in the curves of 1-propenylnaphthalene, Fig. 16, 1-naphthol, Fig. 17, and 1-benzoylaminonaphthalene, Fig. 21. This is because band II is transversely polarized and any substituent which increases the parent pi system's width, B, or percentage contribution of transverse excited structures, C, will have a large effect on band II of A.

A

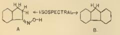
B

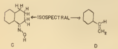
C

3. Fluorene derivatives. Fluorene is a member of the K region sequence, so that aspect of its properties will be discussed in the section of the paper devoted to that sequence.

The absorption spectra of fluorene, acetanilide, styrene and 9,9'-spirobifluorene are compared in Fig. 26. Many compounds such as oxindole derivatives (103), benzaldehyde, acetophenone and cycloalkyl phenyl ketones (195), benzaldoxime and 1-tetralone oxime (247) are isospectral with acetanilide and styrene to a large extent, although it must be remembered that the phenyl ketones have low energy, low intensity n bands also (247). In all these derivatives the common feature is a benzene ring in conjugation with a double bond. This particular combination shifts band II of benzene about 40 mμ and band I about

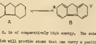
20 mu. There is also an intensity effect on both bands. The spectra of all these compounds resemble fluorene's spectra to some extent, except that the fluorene bands are more intense and further towards the visible. As fluorene is relatively rigid and strained, for a better comparison its spectra should be compared to the spectra of a fairly rigid and strained styrene type compound. In 1-indanone oxime (A) we have such a compound. Examination of the spectral data in the literature (247) shows that this oxime is isospectral with fluorene (B), while 1-tetralone oxime (C) is isospectral with styrene (D).





As all these styrene type compounds are isospectral and iso-pi-electronic with acetanilide, their low energy, low intensity band must be a I band, while the other band is a II band. On the basis of this information the 260 mu band of fluorene is a II band, while the two bands at 288 and 300 mu are vibrational components of the I band. In the benzene-naphthalene sequence the II band was found to be polarised in the transverse direction, for substitution in the 1-position affected this band the most. On the other hand, in fluorene the II band is polarised to a large extent in the longitudinal direction, for strong n→pi transition substituents in the 2-position have a greater B effect on band II than on band I. This effect is large enough to cover the I band of many of the compounds, Fig. 27, 28, 29 and 30. The B effect on band II is shown for many fluorene derivatives in Table XXVIII. The fact that the II band of fluorene is polarised longitudinally is not exceptional for the other members of the K region sequence will also be found to have their II bands polarised similarly.

In reference to the bathochromic effect let us consider longitudinal polarisation. A contributes to the ground state, B to the



excited state. B, is of comparatively high energy. The substitution of any group which will provide atoms that can carry a positive or negative charge more readily than carbon will lower the energy of B and hence permit it a greater contribution to the state of the molecule. If groups are provided that will carry both the positive and

TABLE XXVIII

THE CHANGE IN WAVE LENGTH IN THE II BAND OF FLUORENE CAUSED BY SUBSTITUTION

X	in mμ
Nitro	71
Acetyl	53
Hydrogen	0
Acetoxy	4
p-Nitrobenzenesulfonylamino	10
Hydroxy	12
Methyltosylamino	14
Tosylamino	15
Carbethoxyamino	23
Amino	28
Acetylamino	28
p-Nitrobenzylamino	38
Dimethylamino	43
Benzoylamino	55

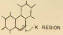
the negative charges more readily than carbon, the difference in energy between A and B will be much less and the bathochromic effect will be even greater. This is borne out by the fact that fluorene has its wave length maxima at 260, 2-aminofluorene at 289, 2-nitrofluorene at 331 and 2-amino-7-nitrofluorene, Fig. 33, at 400 mu.

4. Polyphenyl sequence. For a thorough understanding of the relationship among the diverse individual members of the aromatic carcinogens the assignment of spectral bands must be completed. This means that the reason for every bump, maxima and shoulder in the absorption spectra of a carcinogen must be known for a proper understanding of that carcinogen.

One would expect that the assignment of the 248 mu band in biphenyl would be a simple affair. Woods *et al* (304) have stated that this band is similar to the I band in benzene. The reasons given for the assignment are that the repulsion between the ortho protons and the greater separation of charges in the excited state involving both rings would cause the energy of this excited state to be fairly high. Consequently the absorption of biphenyl would be at shorter wave lengths than would the absorption of benzene. However, on the basis of this line of reasoning, the 248 band of biphenyl could be either a I, II or a mixture of I and II bands. Present day consensus of opinion is that the 248 band is a II band (100, 170). Our assignment of this band as a II band is based on the following reasons. (a) The main band of fluorene, which can be considered a substituted biphenyl, is a modified II band. As this band is longitudinally polarized in fluorene one would expect the analogous band in biphenyl to be similarly polarized. Comparison of Fig. 2 with Figs. 3, 6 and 8 shows that this is true, for substitution of an amino group in the 4 or 4,4' positions causes a large B effect on the II band. (b) Comparison of the high intensity bands of benzene, biphenyl and terphenyl derivatives in Figs. 2, 3, 4, 5 and 6 shows an approximate linear relation. This fits in with the assignment. (c) In the discussion of the K region sequence it will be shown that the I band of all of the members is transversely polarized. This means that substitution in the ortho or meta position to the biphenyl linkage will have a B effect on the hidden I band. On the other hand this substitution in the meta, and especially the ortho, position will decrease the intensity of the II band because of the steric hindrance of the ortho substituent and the electronic interaction of the ortho, or meta, substituent with the ring to which it is attached. Essentially the -I effect on the II band arises from a decrease in conjugation between the two rings. These effects are shown in Fig. 9. Substitution of a 2-acetylamino group in biphenyl causes a -I effect. When an amino group is substituted in the 2-position of biphenyl, the -I effect is larger, while the greater resonance effect of the amino group (as compared to the acetylamino group) causes a pronounced B effect on the I band. The result of a transverse polarization is also seen in 2-hydroxybiphenyl, Fig. 10, where a new low energy I band is formed. This band resembles the low energy phenolic band, just as the I band of 2-aminobiphenyl resembles the low energy band of aniline, Fig. 3. The development of a I band in biphenyl derivatives through ortho or meta substitution has also been demonstrated by other workers (21, 118, 229, 298).

The assignment of bands for the polyphenyl sequence is shown in Table XXIX. Note the new o-nitroaniline bands common to the o-nitroamine derivatives of benzene, biphenyl, terphenyl and dibenzofuran, Figs. 4, 5 and 44. The differences in II bands must be emphasized. For example, the II band of anthracene has a large amount of fine structure, while the analogous band in biphenyl has no fine structure. The reason for this is that in anthracene the energy absorbed is converted into electronic vibrational transitions, while in biphenyl (which is not a 'rigid' molecule) the energy absorbed can 'leak out' more readily causing movements involving the different movable parts of the molecule.

5. K region sequence. A large number of polynuclear hydrocarbons have been found to be carcinogenic (143). The vast majority of these carcinogens contain a phenanthrene ring with the meso position unsubstituted. Because of this fact the phenanthrenic 9,10-position in these compounds has been called the Krebs, or K, region. To



strongly emphasize the importance of the phenanthrene ring a small homogeneous group of polynuclear carcinogens are presented in Table XXX. Pullman (243, 244) has attempted to explain the carcinogenic

TABLE XXIX

POLYPHENYL SEQUENCE BANDS

Compound	I	II	o-Nitro-amine band
	Wave length in mμ	maxima	
Benzene	254	208	
Aniline	284	237	
2-Nitro-1-carbethoxyaminobenzene	274	229	334
2-Nitroaniline	276	231	404
p-Phenylenediamine	291	245	
Biphenyl	—	247	
Benzidine	—	290	
2-Aminobiphenyl	$\underline{300}$*	$\underline{250}$*	
4-Aminobiphenyl	—	275	
2-Hydroxybiphenyl	288	247	
3-Nitro-4-acetylaminobiphenyl	—	260	355
3-Nitro-4-aminobiphenyl	—	268	427
p-Terphenyl	—	278	
4-Amino-p-terphenyl	—	302	
3-Nitro-4-carbethoxyamino-p-terphenyl	—	287	376
3-Nitro-4-amino-p-terphenyl	—	297	431
4,4''-Diamino-p-terphenyl	—	311	

* Underlined 'maxima' are shoulders.

TABLE XXX

PHENANTHRENE CARCINOGENS (143)

Compound	Positions substituted 1	2	3	4	Carc. Activity
Phenanthrene					0
Chrysene	B —	B*			0
1,2-Benzanthracene		B —	B		0
3,4-Benzphenanthrene			B —	B	weak
1,2,3,4-Tetramethylphenanthrene	M**	M	M	M	weak
5-Methylchrysene	B —	B		M	carc.
6-Methylchrysene	B —	B	M		carc.
5,6-Dimethylchrysene	B —	B	M	M	carc.
10-Methyl-1,2-benzanthracene	M	B —	B		carc.
9-Methyl-1,2-benzanthracene		B —	B	M	carc.
9,10-Dimethyl-1,2-benzanthracene	M	B —	B	M	carc.
1-Methyl-3,4-benzphenanthrene	M		B —	B	carc.
2-Methyl-3,4-benzphenanthrene		M	B —	B	carc.
1,2-Dimethyl-3,4-benzphenanthrene	M	M	B —	B	——
1,2,3,4-Dibenzphenanthrene	B —	B	B —	B	carc.

* B — B = fused benzene ring
** M = methyl

activity of such compounds by relating an optimum density of pi electrons in the K region with the activity. Because of the fairly large number of exceptions (14,15,135) to this theory, a thorough study of the chemical and physical properties of phenanthrenic compounds should be undertaken. For that purpose this section of the paper contains a study of the spectral relationships between phenanthrene (which could be considered the parent ring system of the vast majority of polynuclear carcinogens) and the parent ring systems of the carcinogenic amines.

When the K region in phenanthrene is replaced by isosteres, the K region sequence is obtained. The concept of isosterism has been recently reviewed (120). In the K region sequence the n→pi transition for the isosteres gets stronger in the order - - $N_2 < CH_2 < O < S < Se < NH < CH{=}CH$. This order is based on the data in Table XXXI. The bands of biphenyl and fluorene have been assigned in the sections devoted to those compounds. The assignment of the bands in dibenzofuran, Fig. 40, is difficult for here is a compound which has two main bands, one of which resembles the II band of fluorene, Fig. 26, while the other resembles the II band of dibenzothiophene, Fig. 46. On the other hand the I band of 2-hydroxybiphenyl, Fig. 40, coincides with the low energy band of dibenzofuran. A substituent in the para position of dibenzofuran has a B effect on both of the bands, Fig. 42. This would make it seem as if neither band was a I band. On the basis of this information the band at 280 mμ is believed to be a II band containing an enveloped I band. Essentially

TABLE XXXI

K REGION SEQUENCE BANDS

	I		II	III
X	Wave length maxima in millimicrons			
H_2		247		?
CH_2	289 300	260		?
O		280	249	?
S	312 324		285	233
Se	317 328		287	238
NH	323 336		293	234
CH=CH	309 315 323 330 338 346		293	252

this II band is similar to the biphenyl and fluorene II bands. The band at 249 mμ is a new II band similar to the 'finger' band of the remainder of this varioconjugate sequence. The assignments will have to be checked by a study of 1, 2 and 4 substituted derivatives. For studying polarisations, acylamino substituents should be used because of their strong conjugation effect. These substituents should cause transverse polarisation and thus bring the I band into the open.

Comparison of the spectra of dibenzothiophene, Fig. 46, with its 2-substituted derivatives, Figs. 47, 50 and 53, and 3-substituted derivatives, Figs. 47 and 49, shows that transverse polarisation causes a B effect on the low energy band of dibenzothiophene (which, for this reason, is a I band), while longitudinal polarisation causes a large B effect on the 'finger' band at 265 mμ (which, consequently, must be a II band). The assignment of the bands of isospectral dibenzoselenophene, Fig. 55, would naturally be the same as for dibenzothiophene.

The high intensity bands of carbazole, Fig. 46, dibenzothiophene, dibenzoselenophene and phenanthrene, Fig. 55, are all remarkably similar. This high intensity band shows a very weak B effect on transverse or longitudinal polarisation. In many respects these high intensity bands resemble the high intensity bands of naphthalene and anthracene. Thus, it is very probable that the high intensity X region sequence band is a III band.

The assignment of the bands of carbazole and phenanthrene is based on the remarkable similarity of the spectral curves of carbazole, dibenzothiophene, dibenzoselenophene and phenanthrene. The author would like to emphasize that these band assignments are based on the facts at hand, which are incomplete, and so the assignments are considered tentative.

All the tested p-amines of this K region sequence have been found carcinogenic, Table III. On the other hand no extensive studies have been made of the 9,10-dimethyl-1,2-benzanthracene K region sequence. The compound, X = CH=CH , is a powerful carcinogen,

while the compound, X = S, has been found to be moderately active on painting but inactive subcutaneously in mice (14, 290). It would be of interest to test the other compounds of this sequence. In the 3,4,5,6-dibenzphenanthrene K region sequence the compounds,

X = CH=CH, S, O, CH_2 were found to be inactive, while the isostere, X = NH, was found to be a strong carcinogen (143).

The relationship between these polynuclear carcinogens and the carcinogenic amines will be discussed in the next section of the paper.

D. Pi Basicity

The carcinogenic amines and the polynuclear carcinogens are apparently two great distinct groups of carcinogens. Without exception the members of both groups are pi bases. In section IIIA it was shown that both groups react with the different types of acids. As seen in Table Va, the addition of an amine group or a fused benzene ring to a parent pi system will have a B effect of the same order as far as the color is concerned. For example, while naphthalene gives a yellow complex with s-trinitrobenzene, 1-naphthylamine and anthracene form red complexes.

It is very probable that substitution of an amino group or fusion of a benzene ring to a parent pi system will result in an increase in pi basicity. Similarly there are many examples in which the substitution of an amino group or fusion of a benzene ring to an inactive hydrocarbon will impart carcinogenic activity to the molecule. In this respect see section II and Table XXX.

The powerful effect of methyl groups in increasing the pi basicity of benzene has been described recently (85, 197). In this respect the position of substitution was extremely important. Similarly the presence and the position of substitution of methyl groups has a remarkable effect on carcinogenic activity. For example, 1,2-benzanthracene and 1',9-dimethyl-1,2-benzanthracene are non-carcinogenic while 9,10-dimethyl-1,2-benzanthracene is an extremely powerful carcinogen.

The effect of size and shape on the reactivity of pi bases with some reference acid is of decided importance. For example, benzene forms a very weak picrate, naphthalene and anthracene fairly strong picrates while naphthacene and pentacene do not form picrates. In the polyphenyl sequence biphenyl and p-terphenyl do not form picrates, while in the K region sequence all members, except biphenyl, form picrates. On the other hand the author predicts that the 4,4'-dinitropolyphenyls will react with the polyphenyl pi bases and similar compounds. For example, preliminary studies have shown that 4,4''-dinitro-p-terphenyl seems to be a 'specific' complexing agent for the p,p'diamines as shown in Table XXXII.

TABLE XXXII

4,4''-DINITRO-p-TERPHENYL COMPLEXES

	Color	m.p.
4,4''-Diamino-p-terphenyl complex	red	307-309
Benzidine complex	yellow-brown	268-269
2,7-Diaminofluorene complex	red-brown	276-277

It is predicted that compounds, such as 4-amino-p-quaterphenyl, 4-amino-p-terphenyl, 3,7-diaminodibenzofuran and possibly p-terphenyl, p-quaterphenyl, 4-aminobiphenyl and 2-aminofluorene will also form weaker, lighter colored complexes with this new complexing agent.

The aromatic carcinogens show a somewhat similar specificity. Here again size, shape and pi basicity seem to be of great importance. Benzene derivatives are small in area and usually have a low pi basicity. This may be the reason for their lack of carcinogenicity.

In Tables XXXIII and XXXIV the parent pi systems of some carcinogenic amines and polynuclear carcinogens are compared. 2-Naphthylamine, I, is the smallest known carcinogenic amine, while the 1,2,3,4-tetramethyl derivative of phenanthrene, Ia, and the 9,10-dimethyl derivative of anthracene, Ib, are the smallest polynuclear carcinogens. In the same way when the amino group of carcinogenic 2-anthramine, II, is replaced by a fused benzene ring, 1,2-benzanthracene, IIa, and naphthacene, IIb, are obtained. Quite a few monomethyl and dimethyl derivatives of IIa are known to be carcinogenic (143). In respect to IIb, there are no known carcinogens containing the naphthacene nucleus. This is probably because of the strong transverse polarization which causes increased stabilization of the diradical forms. For this reason the naphthacenes react easily with oxygen to form peroxides. Derivatives of III, where $X = H_2$, CH_2, O and S, have been found to be carcinogenic (Table III). The theoretically important compounds, 2-aminocarbazole, III where X = NH, and 2-aminophenanthrene, III where X = CH=CH, have never been tested for carcinogenic activity. On the other hand derivatives of IIIa, X = S and X = CH=CH, have been found to be carcinogenic. This X region sequence is also incomplete for $X = H_2$, CH_2, O, Se and NH have probably never been tested for activity. Derivatives of IIIb, where X = NH and X = CH=CH, have been shown to be carcinogenic. Derivatives of IV, $X = H_2$, and IVa, X = CH=CH, are also known to be carcinogenic. X region sequence derivatives of V, VII and VIII have not been tested. Peculiarly enough Va, X = NH, is a strong carcinogen while $X = H_2$, CH_2, O, S and CH=CH have been

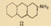
TABLE XXXXIX

PARENT PI SYSTEMS OF THE AROMATIC CARCINOGENS

I

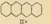
Ia

Ib



II

IIa



IIb

X = H_2, CH_2, O, S

III

X = S, CH=CH

IIIa

X = NH, CH=CH

IIIb

X = H_2

IV

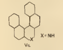
X = CH=CH

IVa

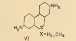
TABLE XXXIV

PARENT PI SYSTEMS OF THE AROMATIC CARCINOGENS

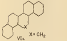
V



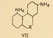
Va



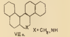
VI



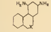
VIa



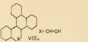
VII



VIIa



VIII



VIIIa

shown to be inactive. A few members of the K region sequences of VIa, VIIa and VIIIa have been tested. For example, VIa where $X=CH_2$, VIIa where $X=CH_2$ or NH and VIIIa where X=CH=CH are carcinogenic.

Examination of the data indicates that a carcinogenic amine can be 'changed' into a polynuclear carcinogen by (1) proper substitution of methyl groups into the parent pi system, (2) change in the K region sequence so that a stronger n → pi transition takes place and (3) replacement of the amino group by a fused benzene ring. It is evident that all three changes affect the pi basicity of a molecule. It is possible that (1) and (2) strengthen the pi basicity of a molecule while (3) weakens the basicity. This should be investigated for here we have three effects which may help to determine the carcinogenicity of the aromatic carcinogens. The optimum π basicity of the carcinogenic amines is consistent with these facts.

On the basis of the facts presented in this paper it seems possible that the pi basicity of the aromatic carcinogens is of great importance. If this is so, then one of the steps involved in this particular type of carcinogenesis is the reaction between the pi base carcinogen and a biological 'acid'. In that case the molecular dimensions and shape of the probable carcinogen will determine its approachability and face to face 'fit' with the appropriate biological 'acid'.

In the development of the theory correlating the carcinogenicity of the azo dyes with their physical properties, section VIB, it was shown that the presence of acidic hydrogen in the molecule destroyed the activity. Examination of the literature (143) discloses that the introduction of a group containing acidic hydrogen into a polynuclear carcinogen also tends to destroy its activity. The absence of acidic hydrogen in pi bases undergoing reaction with a pi acid seems to be of some importance also. For example, p-sulfanilic acid and o-hydroxyaniline do not form complexes with s-trinitrobenzene while ethyl p-sulfanilate and o-anisidine form complexes (282). Pi complexes are known to be decomposed easily by an excess of some hydroxylic solvent. This is probably due to the action of the hydrogen ion. The complexes of pi bases with picric acid are less stable than those with s-trinitrobenzene or 2,4,7-trinitrofluorenone. This is most likely due to the presence of hydroxyl hydrogen in picric acid which becomes acidic in hydroxylic solvents very readily. This instability of the picric acid complexes is also shown by the fact that picric acid complexes of hydrocarbons usually melt lower than the analogous s-trinitrobenzene derivatives (100 examples found in the literature showed no exception to this rule). It has been demonstrated that the fairly basic methylbenzenes combine with a proton to form a complex (197). It is possible that this reaction may be the competing reaction for pi complex formation in hydroxylic solvents. It would be interesting to see how the polynuclear carcinogens would react under similar circumstances for they are probably stronger pi bases. These facts on acidic hydrogen take on greater significance when the statistics of gastric cancer are surveyed (18,84,279). Approximately 50% of gastric cancer patients have achlorhydria and about 40% have a lowered free HCl. In respect to the gastric cancer patients with 'normal' free HCl, it is apparent that part of the stomach could be achlorhydric, while the other sections of the stomach could produce enough HCl to make up the deficit. Attempts to induce gastric cancer experimentally in animals have failed consistently. The conclusion is inescapable. The presence of acidic hydrogen interferes with carcinogenesis.

Examination of the spectral data in this paper - - Table XXVIII, Figs. 12, 14, 16, 18, 19, etc., - - shows that the n→pi transition increases in strength for the isosteric series - - $CH_3 < OAc < Cl < OH < NHAc < NH_2 < SH < CH{=}CH_2$. The remarkable fact about this series is that parent pi systems containing NHAc, SH or $CH{=}CH_2$ are closely isospectral. Some examples are 2-vinylnaphthalene, Fig. 16, 2-naphthalenethiol and 2-acetylaminonaphthalene, Fig. 18, and acetanilide and styrene, Fig. 26.

On the basis of qualitative evidence it has been deduced that pi basicity may be of importance in carcinogenesis. Assuming the strength of the n→pi transition of an isostere is directly proportional to the increase in pi basicity caused by this isostere, then derivatives of 2-vinylfluorene or 2-thiolfluorene should prove to be carcinogenic. This line of reasoning applies also to compounds closely iso-pi-electronic with other carcinogenic amines.

VII

SUMMARY

A. Correlation of the Carcinogenic Activity of the Azo Dyes with their Physical Properties

Although a fairly large number of carcinogenic amines are known, there has been no adequate theory correlating the carcinogenic activity of these compounds with their properties. In this respect a new theory based on experimental evidence is introduced in this paper. Because the chemical environments in the animal body are extremely important, the correlation study was concentrated on the azo dyes of which a large number have been found to cause cancer of the liver in the rat. On this basis the assumption was made that the chemical and physical environments of the hosts were constant and the only variables would be the chemical and physical properties of the orally administered azo dyes. The new theory correlates the carcinogenicity of these azo dyes with their basicity and molecular dimensions. The importance of other factors, such as metabolism and presence of acidic hydrogen, is also discussed.

B. Physical Properties of the Carcinogenic Amines

The general properties of these amines are found to be --

(a) All unsubstituted carcinogenic amines can assume a planar position. Any substituent that increases the thickness of the molecule decreases the activity. (b) All known carcinogenic amines have the amine group is the extended para or, sometimes, the ortho position. (c) Substitution of a group containing acidic hydrogen into a carcinogenic amine decreases or destroys the activity. (d) The pKa in water of the carcinogenic amines is found to lie within the narrow limits of 3 to 5 units.

On the basis of these facts and the results obtained with the azo carcinogens it is evident that the basicity, molecular dimensions and metabolism of the carcinogenic amines are three very important factors which determine the activity of these amines.

C. The Pi Basicity Theory of Carcinogenesis

Based on a fairly large amount of qualitative evidence a new theory is presented which relates the pi basicity of the aromatic carcinogens with their activity. The relationship between the carcinogenic amines and the polynuclear carcinogens is shown. The importance of the molecular geometry of the carcinogens and the chemical and physical environments of the animal body are also stressed. The inhibitory effect of acidic hydrogen in pi complex formation, chemical carcinogenesis and human gastric cancer is consistent with this theory. It is suggested that one of the reactions involved in carcinogenesis may be the reaction between a pi base and a biological acid. The π basicity theory of the azo carcinogens and the optimum π basicity of the carcinogenic amines fit in very nicely with the pi basicity theory of carcinogenesis.

D. Syntheses

1. Over 150 compounds have been prepared, of which more than 45 are new.

2. Qualitative comparison of maleimide, maleic anhydride, crotonic acid, acrylonitrile and acrolein in reactions with several dienes and dienynes brought out the fact that maleic anhydride and maleimide are the most powerful with approximately the same order of activity although maleimide may be a slightly stronger dienophile.

3. A new complexing agent, 4,4"-dinitro-p-terphenyl, has been discovered. On the basis of a study of this compound it was predicted that the 4,4'-dinitro-p-polyphenyls would be found to be complexing agents for the members of the polyphenyl sequence and their derivatives (especially the 4,4'-diamine compounds). This is of theoretical interest because biphenyl and p-terphenyl do not form complexes with the usual pi acids, such as picric acid.

E. Dissociation Constants

The pKa of approximately 23 azo dyes were determined spectrophotometrically in 50% ethanol. For this purpose approximately 100 spectral curves were determined in acid and alkaline solutions.

F. Absorption Spectra

In cancer chemistry there has been an acute need for a collection of spectral curves of the carcinogenic amines. Consequently some 180 spectral curves of carcinogenic amines and allied compounds are presented in this paper. Approximately 40 of these curves were taken from the literature. A large number of band assignments have also been made. The importance to cancer research of sequences, such as the K region sequence, has been emphasized.

The utility of these curves in metabolism, pi basicity and proof-of-structure studies is self-evident.

G. Color Tests

1. A differentiating color test of theoretical interest has been developed for fluorene and its derivatives.

2. An alkaline saturated alcoholic solution of 2-nitrofluorenone has been found to be a fairly sensitive test reagent for enolizable ketones that do not contain acidic hydrogen. A violet to purple color is formed.

3. A 5% pyridine solution of 2-aminofluorene has been found to be an excellent test reagent for aromatic sulfonyl chlorides while a pyridine solution of an aromatic sulfonyl chloride is a fine test reagent for unhindered aromatic primary amines. A dark violet color is formed in both cases. The ramifications of this test have not been fully investigated as yet.

VIII

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